PB-227 988

RESPONSE OF TOLERANT ORGANISMS TO SPECIFIC POLLUTANTS

John L. Blum

Wisconsin University
Milwaukee, Wisconsin

1967

DISTRIBUTED BY:

NTIS

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va. 22151

PB 227 988

050184

REPORT

to the

FEDERAL WATER POLLUTION CONTROL ADMINISTRATION

Response of Tolerant Organisms to Specific Pollutants

Principal Investigator: John L. Blum

Department of Botany

The University of Wisconsin-Milwaukee

Grant Reference No. 1 R01 WP00857

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
U S Department of Commerce
Springfield VA 22151

INTRODUCTION

This study was undertaken to investigate the response of certain so-called indicator organisms to the presence of specific poisons and other substances found with regularity in polluted water, and to determine to what extent this response is dependent on the genetic factor; in other words, to assay the variability of different clones of an organism in its growth response to a pollutant.

For many organisms which grow under polluted conditions there are published reports which indicate a high level of tolerance and others which report a relatively low level. To what extent such apparent conflict is dependent on genetic variability within one species is worth investigation, and the present report is a preliminary attempt to assess the variability of different clones of a single species in this report. A high level of variability among the different clones in their response to a specific pollutant would cast doubt on the legitimacy of using lists of tolerant organisms in the assessment of water quality. If on the other hand relatively little variability is evident in the degree to which growth is reduced by, or death follows, upon prolonged contact with media containing one or more specific pollutants, support would be provided for the concept of organisms as tolerant or intolerant of specific substances or water conditions and for the dependence on such organisms as tools for the measurement of water quality.

By reducing the toxic complex of polluted water to a single frequently toxic substance, it was hoped to simplify the problems raised and to make the results meaningful for a single substance uninfluenced by the presence of other substances at toxic levels. While it must be recognized that the effects of a water pollutant are frequently dependent on the presence of other "unnatural" substances in the water, the present study makes no attempt to assess combined effects of two or more pollutants, but to keep the problem at its simplest level and study variability in the organism as a matter of prime interest, rather than variability of the environment.

THE TEST ORGANISM

In selecting a suitable organism, it was necessary to find one which could be recovered from different fresh-water habitats. It was hoped, furthermore, to locate an alga resistant to conditions found in polluted streams, and one which was recognizable with relative ease. Scenedesmus quadricauda (Turpin) Bréb. was collected with frequency in both polluted and non-polluted habitats, and was furthermore relatively easy to grow in culture. An additional consideration which influenced our decision to select S. quadricauda was the fact that sexual reproduction in this and other species of the genus is unknown except in nitrogen deficient cultures (Trainor and Burg, 1965); cultures which are grown in the presence of adequate nitrogen may, therefore, be presumed to be genetically uniform. Although present

in plankton in certain habitats, Scenedesmus quadricauda also grows on the bottom and is for this reason a better indicator of stream conditions at a given point--if indeed it can be called an indicator at all--than a purely planktonic organism would be. It has been used in many recent investigations of algal physiology and fine structure (Basavskaya and Markarova, 1959; Bisalputra and Weier, 1963, 1964; Castelfranco and Bisalputra, 1965; Osterlind, 1949; Rodhe, 1948; Bisalputra, 1965; Trainor, 1963, 1964, 1965, 1966; Swale, 1967).

Trainor (1964) showed that certain spine-bearing Scenedesmus coenobia could be isolated in axenic culture and could grow to resemble various forms such as S. longus, S. abundans, S. acutiformis or S. bijugatus, within a period of four weeks. As with various Scenedesmus cultures, stable cultures of the four species just mentioned are known to exist and to produce only one coenobial type. Of 27 spine-bearing organisms isolated by Trainor (1966), 24 were found to be stable in culture, whereas 3 of the 27 were pleomorphic, that is to say, they gave rise to cultures consisting in part of unicells, in part of colonies resembling S. longispina, in part of colonies resembling S. semipulcher, and in part of colonies resembling S. quadricauda. That such pleomorphism can be dependent in part on chemical influences is shown by the work of Gistl, (1932). This experimentor exposed Scenedesmus quadricauda to 1/33 n sodium cyanide and observed progressive changes resulting in the loss of the spines of the terminal cells, the

irregular arrangement of the cells of the coenobium, and finally in the adoption of a nearly filamentous organization--numerous cells joined into long chains. Trainor (1963) also reports that Scenedesmus dimorphus, which was isolated from greenhouse soil, grew as typical 4 to 8-celled coenobia only in unialgal, bacteria-free isolation. In the presence of "a soil bacterium," and also in soil extract medium, Trainor was unable to find a single coenobium in the culture; instead, the Scenedesmus grew as unicells.

Scenedesmus quadricauda has appeared on various lists of algae indicative of pollution. For example, Kolkwitz and Marsson (1908) and Liebmann (1951) have both classified it in the β mesosaprobic group of indicator organisms, whereas Šrámek-Hušek (according to Fjerdingstad, 1964) has classified it as an α-β mesosaprobic-oligosaprobic organism. A more modern tendency is probably the conclusion of Fjerdingstad (1964) that Scenedesmus quadricauda is one of the plankton organisms more indicative of eutrophication than of pollution. According to Beck (1954) an indicator organism is a plant or animal, the presence or absence of which is indicative of some fact or facts with respect to its environment. The argument presented in the present report is dependent merely on the fact that the organism has been used at varying times in the past as an indicator of polluted water and as such is one of the organisms whose presence on such lists is worthy of question by the method employed in the present paper. No claim is made that S. quadricauda is in fact a "pollution" indicator.

DESCRIPTION OF CLONES

Of the five clones with which experiment J began, all were essentially typical Scenedesmus quadricauda. There were, however, slight morphological differences between certain of the clones and in some cases these differences did not become apparent until after the clones were in culture for some months. Clones 3 (Fig. 3) and 5 (Fig. 5) are essentially similar in all respects, possessing prominent spines on both terminal cells. Clone 1 (Fig. 1) is essentially similar except that the spines were slightly less stout. Clone 4 (Fig. 4) is similar to 1 but generally appears to have still fainter spines. Strain 2 apparently possesses two types of coenobia. One (Fig. 2c) is essentially similar to strains 3 and 5 in all respects. Other coenobia (Fig. 2a, b) exhibit a longitudinal ridge on the internal cells. This is more conspicuous on certain coenobia than on others. The presence of this ridge was not noted on these cells until they were in culture for some time, but a careful examination of the prepared slides made from strain 2 in 1966 make it clear that such cells were present in the culture from the start. The significance of this apparent pleomorphism is not yet clear. It has been shown (Trainor, 1965) that certain Scenedesmus species are not pleomorphic in media in which other species are pleomorphic (Trainor, 1965). Trainor has commented further on strains of Scenedesmus growing in his laboratory of which most representatives resembled S. bijugatus, but at least 10 per cent of the colonies had longitudinal ridges on all four cells--these coenobia resembled S. acutiformis. Of various algal cultures

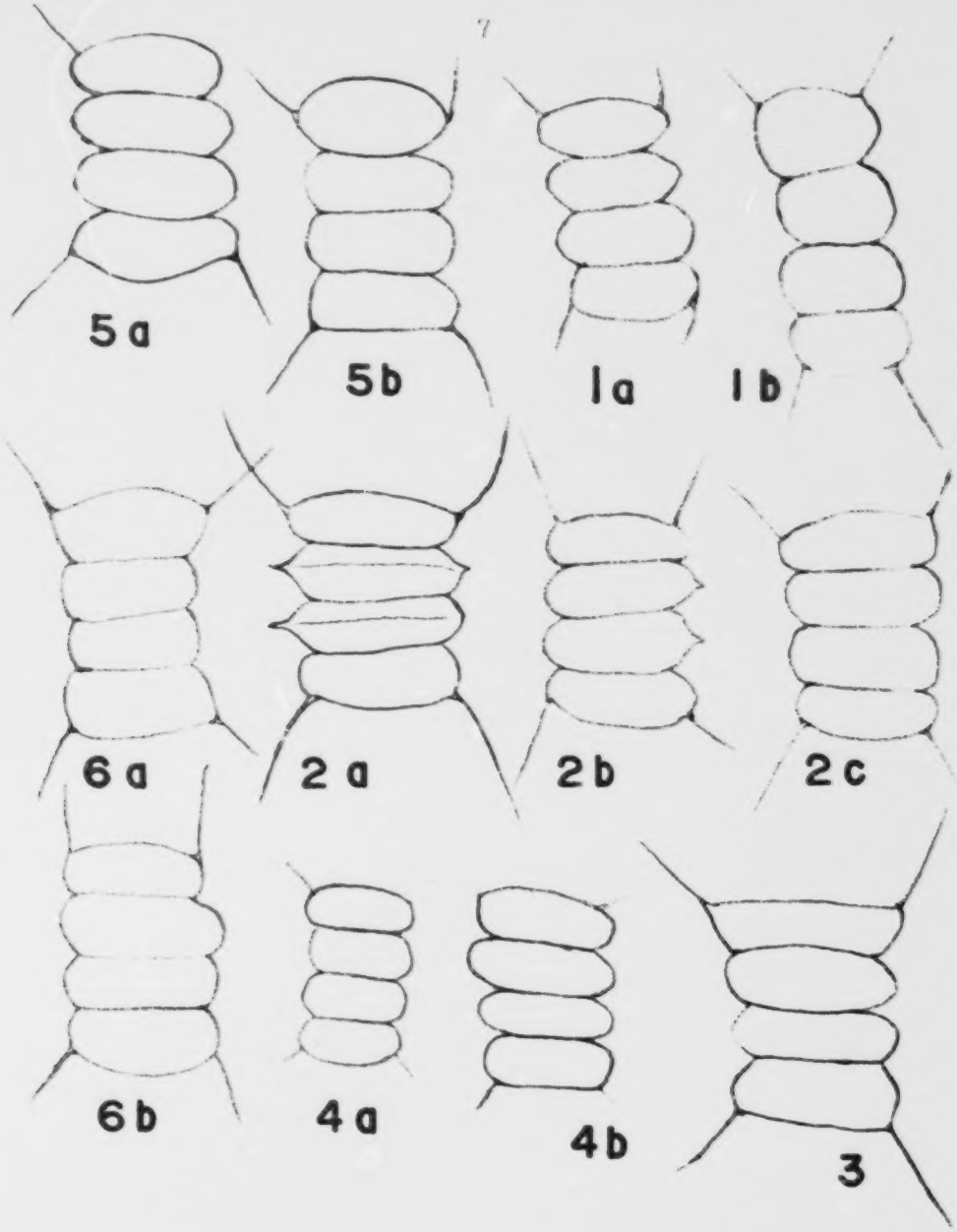

FIG. 1-6. Strain or clone numbers correspond with figure numbers.

studied by Trainor, S. quadricauda (culture 76) showed little variability. In his young cultures, 4- and 8-celled coenobia were produced in almost equal numbers. In older cultures, 4-celled coenobia were more typical.

RELATED REPORTS

The data from Experiment J became the basis for an M.S. thesis by Wm. Woelkerling (1966) who served as research assistant for this project in 1965-66. Experiments K and L were performed largely by NSF Undergraduate Research Participation students, Jayne Freiburger and Carol De Dobay, whose 1966 reports on these respective experiments are listed in the bibliography and filed in the Department of Botany, University of Wisconsin - Milwaukee.

EXPERIMENTAL PLAN

A series of strains of S. quadricauda were collected from a variety of habitats, polluted and non-polluted and isolated in pure culture. From these cultures, clones were established and maintained as stock cultures. The clones were subjected to specific poisonous pollutants at a range of concentrations intended to include both non-toxic and significantly toxic ranges. After a growing period of at least 20 days, the experimental and control cultures were killed and determinations made of population size by various methods. In certain of the experiments performed, determinations of population size were likewise made at regular intervals during the test period. Altogether, seven

such experiments were performed. In all experiments, all experimental tubes were prepared either in duplicate or in triplicate. Following termination of each experiment, visual counts of population size in the respective strains and pollutant concentrations were made. The presence of genetic variability with respect to pollutants present was considered to be demonstrated when a given clone exhibited significant growth at a concentration of a given pollutant which was lethal or algistatic to one or more other strains.

POLLUTANTS USED

The pollutants were chosen on the basis of relative frequency as a pollution "problem", and relative chemical simplicity: on such considerations, complex wastes such as those from refineries, canneries, and chemical industry generally were disqualified. The principal pollutants selected for these experiments were phenol, copper, and biodegradable detergent. Additional experiments involving cyanide and sodium chloride are not reported here. Phenol appeared as a pollutant in 3 experiments (J, M and O) and $CuSO_4$ also in 3 (K, N and P), Experiment L was concerned with detergent.

Growth in a series of concentrations of different chemical substances such as may be interpreted either as pollutants or as nutrient substances has already been carried out with S. quadricauda by Osterlind, (1949). This author subjected Scenedesmus cultures over varying periods

Reproduced from best available copy.

of time to a series of concentrations of sodium, potassium, magnesium, calcium, manganese, iron, silicon, ammonium nitrogen, nitrate nitrogen, phosphorus, and sulphate. While several of these experiments bracketed values which were in the range of insufficient to sufficient for algal growth , none of them bracketed values which were so high as to be clearly injurious. Sorensen (1950), reported on experiments in which unspecified algae were grown in various concentrations of industrial wastewater with a high content of sodium, hydrochloric acid and phenol. This wastewater was diluted to various degrees and the survival rates of algae and Daphnia were observed over a 12-day period.

MEDIUM

For this work, Bristol's Medium (Chantanachat and Bold, 1962) was employed as a medium for algal growth for stock, build-up and experimental cultures. This has proved to be an excellent medium for Scenedesmus and other algae which are intended for similar experimental treatment. For this reason, Bristol's medium was used throughout in spite of the lowered toxity exhibited by copper in the presence of EDTA, (Fitzgerald and Faust, 1965) which is one of the Bristol's components.

MATERIALS AND METHODS

1. Sources of clones: Samples of water were taken from numerous habitats in New York, Pennsylvania, Michigan, and Wisconsin. After collection, these were subjected to careful examination for the presence of S. quadricauda. In any sample which contained colonies resembling this species, a number of coenobia were selected and planted on Bristol's agar. Of numerous colonies so isolated, six were eventually used for experimental purposes. The others were rejected for different reasons, in part since they were suspected of being other species. One of the six strains ultimately utilized was obtained from the culture collection of algae at Indiana University--Scenedesmus strain #76. The sources of the individual strains used are as follows (the numbers employed are those which will be used as strain = clone numbers throughout the report).

1. Milwaukee River, Milwaukee County, Wisconsin
2. Cedar Creek, Ozaukee County, Wisconsin
3. Sugar Creek, Walworth County, Wisconsin
4. Cayuga Creek, Erie County, New York
5. Strain 76, Culture collection of algae, Indiana University, Bloomington, Indiana
6. Rock River, Jefferson County, Wisconsin

Of the above streams, Cedar Creek is a tributary of the Milwaukee River which is itself a tributary of Lake Michigan. Sugar Creek is a tributary of the Fox River, which is a tributary of the Illinois River and thus of the Mississippi. The Rock River is a direct tributary of the Mississippi. Cayuga Creek is a tributary of Buffalo River, which is a direct tributary of Lake Erie and thus of the St. Lawrence system. Of these streams, Cayuga Creek can be described as highly eutrophic and moderately polluted

by domestic and agricultural wastes. The Milwaukee River at the point of collection was likewise moderately polluted by domestic wasts, but lacked the extreme level of eutrophication characteristic of Cayuga Creek. The habitats from which samples were taken to provide strains #2, #3, and #6 are only slightly polluted. All of the streams drain regions of moderate agricultural activity and it is to be expected that variable amounts of farm nutrients and wastes are sometimes borne by each of the streams.

The establishment of clonal cultures from each of the streams was necessarily preceded by suspension of a colony or a portion of the colony in a drop of sterile water. Dilution was prepared from this drop by removing a small portion of the suspended material and placing in another drop of sterile water on a depression slide. With the aid of a binocular microscope at 100x magnification, the individual coenobia were picked out one at a time with a fine bore capilary pipette and planted on the surface of fresh Bristol's agar in screw-cap culture tubes. These tubes were placed in a controlled environment room at approximately 350 footcandles of white fluorescent light at 16-18°C. An 18-hour light, 6-hour dark cycle was maintained throughout the study. Purification was accomplished principally by the methods described in Chantanachat and Bold (1967): particularly the use of "multidiscs" and of ultraviolet radiation was found useful in eliminating bacterial contaminants of algal cultures. As a practical measure, in the use of multidisc plates, algal colonies growing on the plates were carefully examined in the vicinity of multidisc points for apparent purity--as evidenced by the apparent absence of bacterial colonies in their vicinity. Colonies which appeared devoid of bacteria were picked under aseptic conditions with a pointed platinum needle and transferred to fresh tubes of Bristol's medium for further growth. After a growth period

of several days, samples of culture were removed with a sterile pipette under aseptic conditions and subcultured in a series of new tubes of nutrient broth and dextrose-yeast media. Scenedesmus was found to grow successfully in both these media, and the presence of bacteria was usually evident after only a few days by the characteristic cloudy condition of the liquid upon shaking. Irradiation with ultraviolet was carried out in closed plastic petri dishes at a distance of 1-3 inches from an ultraviolet source for varying periods of time. After growth of several days, surviving colonies of algae were fished from the surface of the agar plates thus exposed and subcultured in similar fashion in nutrient broth and dextrose-yeast media. By this means a series of axenic cultures were established and maintained throughout the period of investigation. Contamination, however, was frequent and difficult to eliminate. None of the experiments performed was carried out without a certain amount of contamination, which sometimes rendered an entire strain (consisting of 20-30 tubes) unreliable and in some cases useless.

Much of the experimentation was devoted to finding suitable means of inoculating--under laboratory conditions in our building--to permit high levels of successful axenic inoculation. Although an elaborate technique involving a sealed plastic chamber with armholes and rubber gloves for operator and assistant was finally adopted, we were unable to eliminate this problem. The method adopted for sterilization of supplies, pollutants, culture medium, etc. and inoculation into experimental tubes is described herewith:

OUTLINE OF METHOD

Scenedesmus Growth in Pollutant Dilutions

Supplies Needed: (per 2 simultaneous experiments)

2 A flasks, volumetric, 1000 ml. (for stock solution of pollutants)
6 B flasks, volumetric, 1000 ml. (for pollutant dilutions)
3 C flasks, volumetric, 500 ml.
8 D flasks, Erlenmeyer, 500 ml., with side arm
6 E flasks, 250 ml., nephelometer type
4 F flasks, Erlenmeyer, 125 ml., for Bristol's additives
8 G flasks, 500 ml., Erlenmeyer, each with a magnetized stirring bar inside
2 H flasks, Erlenmeyer, 500 ml., (for sterile water--one as a spare)
3 I flasks, Erlenmeyer, 500 ml., (for medium)
8 Millipore filter sets with clamps and covers: filter pore diam. 0.45 u.
194 Test tubes (assuming that six strains, two pollutants will be used with all experimental inoculations made in triplicate)

(n = pollutants (2) X (number of separate inoculations (3) X each concentration (4) X each strain (6)) + (controls (4) X number of strains (6)) + (number of check media for presence of bacteria (2) X number of strains (6)) + (number of pollutants (2) X number of dilutions (4)) + 6 (for subculturing each strain))

2 X (3 X 4 X 6) + 24 + (2 X 6) + (2 X 4) + 6 = 194

10 graduated pipettes, 10 ml. (4 spares)
10 graduated pipettes, 1 ml.
2 pipette cans, stainless steel
1 beaker, 2000 ml.
2 cans of peracetic acid spray
1 can baby powder
3 syringes with sinker and automatic measuring attachment
5" sq. aluminum foil in 6-inch petri dish
2 transfer chambers, 1-man type and 2-man type
12 test tube racks
2 rubber-glass siphons in rubber stopper for 500 ml. flasks
70% ethanol
Touch-o-matic burner
Large (3 gallon) plastic bags
Suction pump
Containers for waste
Felt Pencils
8 cotton stoppers, for G flasks (incl. 2 spares)

I. Preparatory Growth of Cultures
   1. Prepare subcultures in flasks E of the respective strains of algae about one month prior to zero day (principal inoculation date).
   2. Immediately prior to zero day, remove with a sterile pipette an aliquot to count with haemocytometer. Determine the dilution

factor (for each strain) necessary to obtain 8 X 10^4 coenobia/ml. in each.

or

1. Using algal cultures growing for about one month prior to zero day in flasks E (with side arm continued into a standardized round glass tube (13 ml. o.d.) measure periodically over a growth period of several days or weeks, optical density at 600 u. (scattering), with the B & L Spectronic 20 colorimeter.
2. When optical density has been reduced to 20% transmission, transfer cultures to "hold" conditions (reduced light at 4 degrees centigrade).

II. Preparation of Dry Supplies

3. Label all test tubes:
   1-6 "strain" numbers
   I (phenol), II ($CuSO_4$)--pollutant
   A (control)--E (greatest concentration of pollutant)
   examples 3IIC, 4IA
   Label all volumetric flasks for pollutant concentrations
   Label all D flasks
   Label all G flasks (with magnets)
   Label each rack with conspicuous color code
4. Sterilize in individual wrappings: pipettes in can, six 100 ml. graduates, rubber stoppers for D flasks, foil (twenty 5-inch squares in petri dishes), syringes, racks with test tubes, containers for waste, extra cotton stoppers for D flasks, Millipore filter assembly sets, with papers and cups separate and funnels in place on D flasks; Seitz filter.

III. Preparation of Tubes for Sterility Test

5. Prepare 500 ml. each of nutrient broth (nb) and sucrose yeast (sy) media.
6. Deliver to labeled tubes, 10 ml. per tube, one tube for each medium for each algal strain (6) and one for each pollutant dilution (8), twenty tubes in all.
7. Cap, wrap, and sterilize tubes.

IV. Preparation of Algal Dilution

8. Sterilize six 500 ml. flasks (H) of distilled water in autoclave.
9. Prepare small transfer chamber (hard plastic front) and place cultures, G flasks and magnets, flasks of water (H) and graduates inside.
10. Using previously sterilized graduate or pipette, deliver measured, necessary amount of culture and of distilled water to G flasks to bring the contained culture to 80,000 coenobia per ml. At least 400 ml. of this dilution should be prepared. Cap flasks and cover with foil.

V. Preparation of Pollutants

11. Make up stock solutions of pollutants, using volumetric flasks.
12. Make (and label) needed pollutant dilutions in volumetric flasks (A, B, and C).

13. Spray inside of small transfer chamber (hard plastic front). Place sterilized and wrapped D flasks with Millipore funnels immediately to left of chamber, but outside it.
14. Load the chamber so as to contain:
    A. Sterile petri dishes with Millipore filter papers
    B. Sterile forceps in package
    C. Petri dishes with sterile aluminum foil
    D. Burner (previously immersed, together with its gas hose, in 70% ethanol and then allowed to dry inside the chamber)
15. Procedure for Millipores: Two people are needed for this (L and R).
    L. readies top (cup) and bottom (funnel) by partially unwrapping just outside the chamber.
    R. (working in the chamber) opens forceps package, opens petri dish.
    L. opens bottom paper wrapper outside chamber but immediately introduces bottom part into chamber.
    R. flames forceps and immediately places and centers filter disc on wire or glass surface.
    L. immediately opens cup paper wrapping and introduces cup into chamber.
    R. places cup in position and clamps it, then removes assembly from chamber.
16. Sterilize pollutant dilutions by filtration through sterile Millipore filters into 500 ml. flasks with side arm (D), using a suction pump with large backwash flask. Also sterilize with Millipore or Seitz filter any medium additive which may be destroyed by autoclaving, by similar filtrations into sterile flasks.
17. In the chamber, remove filter glassware from flasks (D). Flame and cap with sterile rubber stopper, cover with aluminum foil.

VI. Preparation of Medium
18. Prepare and sterilize large flasks (I) of freshly prepared Bristol's medium containing 500 ml. each; after autoclaving, add, using transfer chamber, any necessary additives previously sterilized in Seitz or Millipore filter.

VII. Preparation and Loading of Two-man Transfer Chamber
19. Inflate and purge out with peracetic acid large (soft plastic) transfer chamber, with interior door in place. Leave spray can inside. A magnetic stirrer should be placed below the chamber, in a central position, and in close contact with the plastic floor of the chamber.
20. Swab down flasks D (pollutant dilution) and flasks G (algal dilution) with peracedic or alcohol. Cover with sterile plastic bag and place in port.
21. Put outer port door in place. Spray port area thru port holes. Replug port holes. Remove inner port door.
22. Place flasks D and G in chamber and replace inner port door.

23. Remove outer port door. Place remaining supplies, grouped for convenience, in large plastic bag which can be sprayed inside, before each delivery to the chamber is made, and then sealed to the open port while objects are transferred to the chamber. Before each such transfer, the inner port door must be removed from inside, and the port area sprayed from the inside. Proceed in this way to introduce into chamber wrapped syringes, two rubber tubes, all test tubes in sterile racks, sterile containers for waste, extra cotton stoppers for flasks G, 500 ml. of 70% methanol in large metal container or two-liter beaker, large flasks of Bristol's medium (I).

VIII. Inoculation Procedure (Fig. 7)

24. After checking contents of chamber, proceed to unwrap syringe and its rubber siphon tube. Set syringe to desired amount of Bristol's medium per tube (3-6 ml.). Uncap Bristol's flask and place glass-rubber siphon in flask.
25. The following procedure requires two workers:
    L. holds and uncaps tubes, in order.
    R. holds syringe and delivers desired amount into each tube. Between each pollutant dilution syringe and tube must be thoroughly washed with ethanol and repeatedly rinsed with the new dilution to be delivered. Same procedure must be followed between different algal strains.
    L. replugs and replaces tube.
26. Repeat this process for B, C, D, and E tubes with respective dilutions of: pollutant I, then of pollutant II (1 ml.).
27. Repeat this process (for all A tubes) with sterile distilled water to provide blank in compensation for the pollutant dilution present in the other tubes.
28. Place algal dilution flask on magnetic stir and turn on the stirrer. Repeat, as for pollutant dilution with respective dilutions of the algal strains 1, 2, 3, 4, 5, and 6 (1 ml. each), and controls and subcultures.
29. After inoculation is complete, immediately add three drops of formaldehyde to one A (control) tube for each strain of algae being tested.
30. Tubes with formaldehyde are labeled † (= killed) and set aside. All other test tubes are placed within 2 hours in environment room.

Following the inoculation procedure, a series of control tubes were killed with formaldehyde and set aside for counting at an early opportunity. All the other tubes, consisting of the principal experimental and control tubes as well as the tubes to test for presence of bacteria were placed in an environment chamber at constant temperature. Tubes were placed in the light in a slanting position and, except for those tubes designated for bacterial tests, were not removed until the end of the experiment.

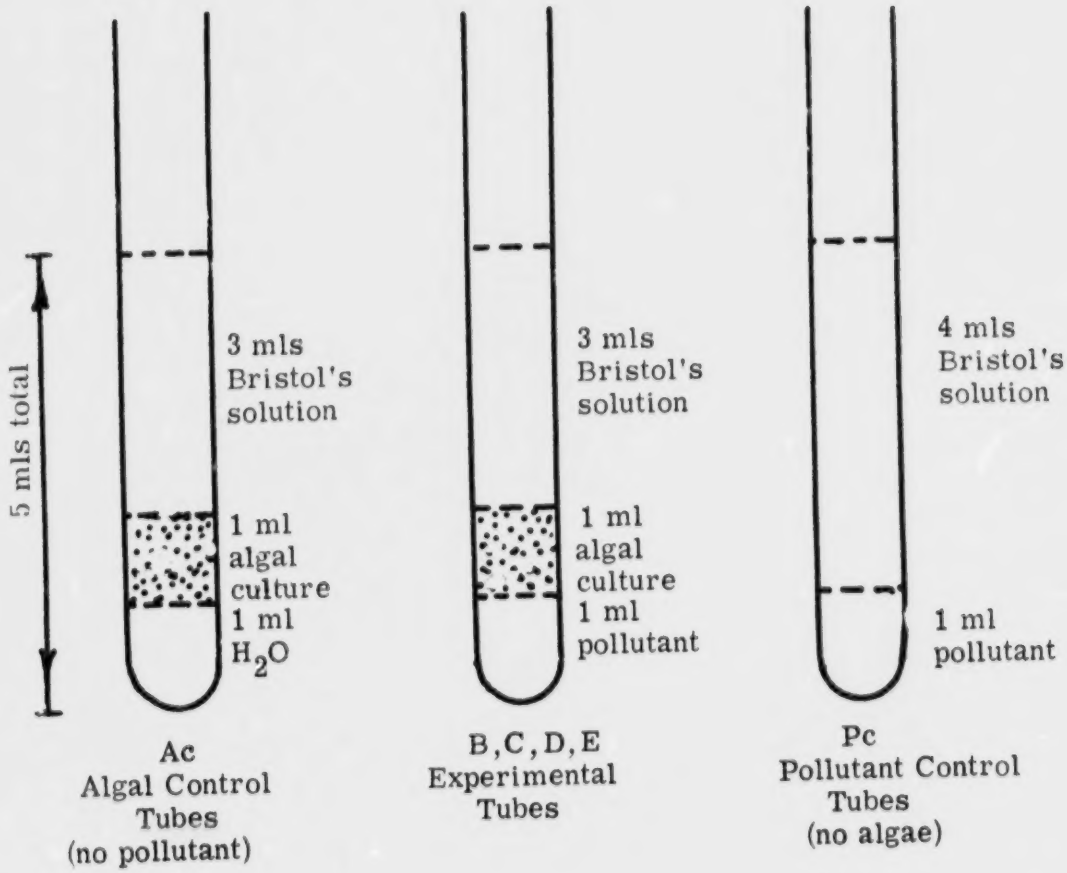

FIG. 7. Composition of algal control tubes (Ac), experimental tubes (B,C,D,E) and pollutant control tubes (Pc), assuming a total volume of 5 ml/tube.

Numerous parameters can be employed to measure the growth of coenobial planktonic algae such as S. quadricauda. Rodhe, in his classic paper (1948), on the environmental requirements of freshwater algae, indicated that "substance production, cell multiplication, and chlorophyl formation are rather independent aspects of the development of a culture. Their courses are affected in different ways for a deficiency of, e.g. nitrate. Parallel determinations should therefore, if possible, be carried out of extinction, cell concentration, and chlorophyl content." The parameters, cell concentration, chlorophyl content, and cell volume were determined in the earliest series of experiments which were made with S. quadricauda. Subsequently, chlorophyl content and cell volume were abandoned as routine procedures and extinction values were determined using scattering light at 660 mμ.

At the end of the experimental procedure--which varied somewhat with the different experiments performed--the tubes were removed, the experimental cultures killed by formaldehyde, and readings were made of population size and any other parameters which were to be determined. For Experiment J, numerous measurement dates were established, and observed throughout the experimental period, The other experiments involved only two measurement dates, zero day (or day of inoculation) and the day of termination of the experiment. In addition to the variation in the length of the individual experiments, which was decided on for various reasons, partly because the cultures grew faster in certain of the experiments than in others, there was variability among the different experiments as to the types of tubes employed. A difference in growth rate exhibited by the various strains as well as a difference in growth rate of one strain when different experiments were compared is apparently

partly due to the fact that the capping of the test tubes was not the same in all of the experiments. The earliest series of experiments employed a plastic screw-cap. In the first of these experiments the tubes were capped tightly with the covers screwed down to prevent gas exchange. The final series of experiments (M-P) involved the use of snap-on plastic tops which permitted free gas exchange between test tubes and the air of the environment room. In general, differences between experimental procedure among the different experiments were adopted in an effort to control bacterial contamination. In other words, uniformity of experimental procedure was sacrificed in the desire to optimize bacterial control. The methods used to measure growth were as follows:

1. Chlorophyl *a* was found to be difficult to measure because of the resistance shown by Scenedesmus to extraction of its pigments. Under the best method of extraction we developed, a small portion of the pigments clearly remained in the cells. Hot and cold methanol, acetone, benzene, ethanol, and methanol-petroleum ether mixtures were all relatively unsuccessful in the removal of residual pigment from the cells, even after mechanical disruption by grinding with sand and dry ice. Using a standardized procedure, chlorophyl measurements were made on the assumption that roughly the same percentage of chlorophyl would be extracted if the procedure were routinized:

   (1) Aliquots consisting of 5ml. from each test culture to be analyzed were frozen and stored in darkness until the time of analysis.

   (2) The samples were thawed, centrifuged and supernatant water decanted.

   (3) Chlorophyl was extracted in 8 to 10 ml. of hot, absolute methanol for 30 minutes.

   (4) The methanol mixture was centrifuged at 6,000 rpm. for 2 minutes.

   (5) The extract was decanted into a second centrifuge tube and the volume of the liquid brought to 10 ml. with addition of absolute methanol.

(6) An aliquot of the extract was measured with a Beckman DU spectrophotometer by the method of Richards and Thompson (1952). Chlorophyl a was computed from the formula:

$$\text{chlorophyl } a = 15.6E_{665} - 2E_{645} - 0.8E_{630}$$

where E is the DU reading obtained for the wavelength indicated by the subscript. Results are expressed in mg/l. (Table 8).

(2) Population volume was determined using a Bauer-Schenk sedimentation tube (Castelfranco and Bisalputra, 1965).

(3) Population counts were obtained using a Neubauer haemacytometer (Lund et al , 1958; Pratt, 1940; Winokur, 1948). In the early experiments, J, K, and L, 25 counts of cells from each culture analyzed were recorded and averaged. In later experiments, M-P, in the interest of including additional clones and pollutants, this number was reduced to 10 counts per culture analyzed. Samples containing dense populations were diluted before counting (Lund et al., 1958).

In the haemacytometer procedure the cover glass and the slide were both cleaned (in 95% alcohol) and dried and the cover glass placed in position on the slide. A finely drawn pipette kept in 95% alcohol was removed and emptied of its alcohol. The tube to be tested was taken in hand and given 25 shakes. The cap was then removed and the pipette immediately placed $\frac{1}{2}$ way down in the tube. Liquid from the culture was allowed to flow into the pipette by capillary action. The pipette was then removed from the tube, the cap of the tube replaced, and the point of the pipette immediately inserted into the V cut of the haemacytometer slide so as to allow the liquid to flow by gravity from the pipette, than by capillarity onto the slide, under the cover glass. After the upper area of the slide was filled in this way by gravity flow, the pipette was removed and placed in the opposite V in the same way. The pipette was then put aside and not subsequently used on that day.

Counts were made under low power of the compound microscope, although high power was occasionally used for study of doubtful colonies or coenobia. The grid closest to the operator was counted first, then the far grid. The 9 smaller squares of each grid were counted in a pre-arranged order by each operator, and insofar as possible, all details of the counting procedure were standardized among the assistants involved in the counting process.

Individuals making counts used mechanical hand counters and counts were entered immediately on a specially prepared sheet which was used both for recording counts and for calculations made to provide a reading in terms of cells or coenobia per ml. (see Appendix). For extremely high concentration of cells, dilutions were generally made. This was necessary for A (control) tubes as well as for the B and sometimes the C tubes. At least in the later experiments, the D and E tubes did not require dilution.

As a general rule, when coenobia were fewer than 500 per grid (of 9 squares) the counts were made without dilution, but when this number was exceeded, dilutions were usually made before counting with the haemacytometer. If during the counting process drying of the culture material under the cover glass occurred before beginning the far grid--evidenced by the enlargement of air spaces at the edges of the cover glass, a droplet of distilled water was added at the edge before proceeding with the counting. If this occurred during the making of an individual count to such an extent that air approached the grids themselves, the results were rejected; then the process of counting started over again. In certain clones of the later experiments all three of the triplicate tubes were counted in this way, but, depending partly on the available time, in other clones, only one tube per pollutant concentration was counted. For certain of the experiments in which many or most of the tubes of a strain were contaminated by bacteria or by fungi, the entire experimental series was rejected. In other cases, dependent partly on the apparent severity of contamination and the absence of axenic tubes in the triplicate series, a contaminated tube was counted. Counts which are based on contaminated tubes are represented in the bar graphs (representing growth in the respective experiments) by shaded bars.

In counting coenobia with the haemacytometer, all coenobia were counted any part of which were found in the area of the grid. Only living coenobia, or coenobia which were apparently living at the time of termination of the experiment, were counted. Empty shells of coenobia were therefore not counted. Two living cells in an otherwise empty 4-cell coenobium shell were recorded as a 2-celled coenobium. Coenobia apparently consisting in low power view of only 1 cell were recorded as 2-cell coenobia since experience made it clear that these were frequently 2-celled coenobia seen from the side. Cell masses which formed in certain of the experimental tubes under conditions of contamination, apparently consisting of short and somewhat branched filaments of cells, were counted as if they were made up of 4-cell coenobia; that is to say, a group of approximately 16 such cells was recorded as four 4-celled coenobia.

4. In the later series of experiments, special nephelometer flasks were employed to measure the growth of individual cultures without opening the flasks. Scattering was measured at 660mμ . with the Spectronic 20. This instrument was also used on sampling days for estimation of population density, likewise by scattering.

For all of the earlier experiments, slides were prepared on each test date of all of the control and experimental cultures. The preparation of permanent slides presents numerous problems, which were encountered and reported by previous workers (V. Johansen, 1960) as well as water dilutions of Karo and of Hoyer's solution (Anderson, 1954) were tried with varying success. The following procedure, derived empirically, was found suitable for staining Scenedesmus for temporary mounts. In Experiments J, K, and L,

sample tubes of all strains and all concentrations of pollutants used were employed in the manufacture of slides, which were prepared in quintuplicate for each strain and concentration:

1. Centrifuge a small aliquot of the culture. Decant.
2. Fix for 1 hour in FAA. Centrifuge, decant and rinse twice with distilled water.
3. Stain for 1 hour with 1/5000 methylene blue in water, centrifuge, decant and rinse twice with distilled water.
4. Place a drop of the solution containing the fixed and stained organisms on a clean glass slide. Allow the moisture to evaporate.
5. Mount in 2/3 dilution of Hoyer's solution.

This method was successful in the preparation of slides intended for observation within the 4 to 5 months following their manufacture. These slides have remained usable for a maximum period of 2 years, but after a few months the methylene blue fades.

Statistical Methods Used

Cell Measurements for Experiments J, K, & L

Length and width measurements were made on randomly selected coenobia 24 hours (approximately) after the manufacture of the slides. These measurements were not repeated in Experiments M through P. From a study of a condensed Romig table (Simpson, 1950) it was concluded that a minimum of 35 length measurements and 25 width measurements were necessary to ensure statistically valid mean values. Length and width of 40 coenobia were measured at each testing interval in experiments J, K, and L. For the purpose of calculating coenobium volume, the coenobe was assumed to be a rectangular solid. Volume was then determined using the formula V = LWH where L = coenobe length. W = coenobe width, and H = 6.22, a thickness constant. This constant is an average of 40 measurements of coenobe thickness determined from living plants. Other investigators (AFHA, 1960; Kutkuhn, 1958) have employed similar procedures in calculating the volume of organisms.

Treatment of Raw Data

The data sheets (Fig. 27, Appendix) were used for totaling units (coenobia) counted under the following categories: 2-celled, 4-celled, 8-celled. Counts were averaged and the results summarized in terms of coenobia per ml. on master sheets, one for each strain per concentration per experiment. Since these were counts of coenobia, one entry in the 2-cell column recorded a coenobium of only half the average volume of one entered in the 4-cell column. Adjustment of these values was made in a second series of master sheets, wherein the 2-celled totals were divided by two and the 8-cell totals multiplied by two: This gives essentially the number of cells divided by 4. The population count sheets (Tables 10-16) given in the Appendix refer therefore to numbers of 4-cell equivalents rather than to numbers of coenobia present. Following the totaling in this way of 2-cell, 4-cell and 8-cell units, the percentage of each of these categories in the total population was determined for each culture tube counted. These percentage totals are likewise given in the Tables 10-16. Population totals were derived from the counts made at the termination of each respective experiment. Bar graphs, representing experiments N through P show the growth made in the respective pollutant concentrations and appear in Figures 14 through 20.

Following the preparation of population counts, a series of summary sheets were prepared (Tables 1-7) on which were entered principal facts and parameters involving each of the different experiments as well as total counts at zero day and at the termination day of the experiment. The increase factor shown by each strain of algae in different concentrations of pollutant as well as the increase factor reduced to a per day basis, are also entered on the summary sheets. The diel increase was used

in the preparation of a series of summary graphs (Fig. 21-26) which illustrate the amount of growth shown at different concentrations of pollutant by the various strains. Data showing growth achieved by the respective strains in all pertinent experiments were assembled to prepare these graphs. (Table 17, 18).

The entire range of experimental and measurement procedures is summarized and illustrated in the flow chart, Figure 8.

RESULTS

Size, Volume and Coenobium Structure

In experiment J, strain 1 was selected for studies of coenobium size and volume when growing in different concentrations of phenol. The average length of coenobia was found to remain nearly constant throughout the test period. At any one time average values never differed by more than 1.5 μ an insignificant difference. The extremes in mean length were 15.4 to 16.9μ . Individual 4-cell coenobia as long as 20.8 μ and as short as 13.0μ were observed. Both length and width of coenobia remained fairly constant throughout the 60-day test period in this experiment. The lowest mean value for coenobium width, 9.7μ , was not significantly different from the highest mean value, 10.7μ . Individual coenobia as wide as 13.0μ and as narrow as 7.8μ were found.

Initially the mean volume of 4-cell coenobia showed a range of 1165.2 to 1113.4 μ^3 At the start of experiment J the volume of the population was 0.33 cc/l in all of the clone 1 cultures (Fig. 12). The volume of plant material increased steadily and in all but one case reached its peak by the 45th day. After sixty days the volume of material showed a range of 3 33

to 6.67 cc per liter.[1] The pattern of development of the total population of plants is shown in Figure 9. The initial range of the population was 7. to 8.6 coenobia per 10^{-4} milliliter and population growth remained in the exponential phase from 0+15 to the end of the test period. After sixty days, coenobia had increased by at least a factor of 36. Final range was 260.9 to 312.2 coenobia/10^{-4} ml. The populations of all strains were routinely analyzed from a percentage standpoint to determine the fraction of total population consisting of two, four, and eight-cell coenobia. Composition of the population in terms of the numbers of these different coenobia fluctuated widely at different times in one experiment, within the same strain, from experiment to experiment, and between different strains. It was usual to find 1 to 25% of the coenobia to be 2-celled, approximately 50 to 90% 4-celled, and approximately 1 to 10% 8-celled. However, a distinct change commonly occurred in per cent composition of these respective types of coenobia between the date of inoculation and the subsequent sampling dates of the respective experiments. In experiment M, for example, strain 5 at zero date had essentially no coenobia of the 2-cell variety, approximately 33.8% of the 4-celled variety, and 66% of the 8-celled variety. At the end of the experiment these values had been changed to 10% 2-celled, 70% 4-celled, and 19% 8-celled; in other words, a sharp reduction in the 8-celled variety and a corresponding increase in the 4-celled variety. Such changes were commonplace. However, within the same strain it was usual to find essentially the same proportion of coenobia of the different types in all of the pollutant concentrations. In experiment M, strain 5 mentioned above, for example, the 2-celled coenobia represented in the respective pollutant concentrations 10%A, 11.6%B, 3.4%C, 1.0%D; the 4-celled coenobia represented respectively 70.6%A, 77.2%B, 67.5%C, 73.1%D; the 8-celled coenobia represented respectively 19.2%A, 11.0%B, 29.0%C, 25.8%D. Strain 3 deviated

---

1. All readings are subject to the 1.33 cc/l error of a Bauer-Schenk sedimentation tube.

consistently from the expected percentages given above for 2-celled, 4-celled, and 8-celled coenobia. Regularly from 30 to 90% of the coenobia in strain 3 cultures were 2-celled, and a sharply reduced number, 20 to 60% were 4-celled. Strain 3 was likewise different in that few if any 8-celled coenobia were present in the cultures. Tables 10-16 give the percentages of different types of coenobia in the respective populations. These are based on the same adjusted totals given in the other tables, and the percentage values therefore represent the percent of total cell numbers contained respectively in 2-celled, 4-celled, and 8-celled coenobia rather than the percentage value of these respective types of coenobia in the population. During the two and one-half years in which the experimentation was carried on, certain of the strains exhibited what appeared to be changes in cells per coenobium; for example, strain 4 in 1966 exhibited high values, frequently approaching 90-95%, of 4-celled coenobia in the population. In the later experiments carried on in 1967-68, strain 4 had a much higher percentage, approaching 60-70% of 2-celled coenobia and the percentage of 4-celled coenobia was reduced to the 40-50% level. The other strains by and large did not show any marked changes over the period of time that they were in culture. However, all of them exhibited apparently erratic behavior on occasion as is shown from Table 19, representing the range of percentages of the respective types of coenobia in the populations of different experiments.

Morphological Variability

Numerous cultures in the course of the different experiments exhibited unusual cell types, in some cases high percentages of cell types other than those expected in typical Scenedesmus quadricauda cultures. However, no clear morphological differences based on concentration were observed during the course of the experiment except for an apparent enlargement of cells

in lethal concentrations of certain pollutants. Within the range of concentrations which permitted growth statistically valid differences in cell shape were not observed. In certain cases where peculiarities in the appearance of coenobia appeared to be characteristic of the tube or of the culture it was generally found associated with bacterial contamination (cf. Trainor, 1963) and was not ascribed to the presence of the polluting substance.

Growth Pattern of Cultures

In Experiment J., various methods of estimating growth of the cultures were employed and sampling dates were fixed at frequent intervals throughout the sixty-day period. Results of visual counts of coenobia in the population (Figs. 9, 10) as well as chlorophyl measurements (Fig. 11) and of population volume (Figs. 12, 13) suggested that linear growth could be expected at least between approximately 15 and 45 days. While some cultures showed good growth after 45 days, (Tables 8-10) a number of them exhibited a decline in growth rate or an actual decrease in coenobium number. For subsequent experiments the growth period was reduced to 48 days or less. Certain cultures clearly grew faster than others, but growth periods between 30 and 48 days were assumed to fall within the linear growth period of all the strains. Experiments performed subsequent to Experiment J. thus employed only two test dates, the zero date and the terminal date of the experiment. In Experiment J. (Table 1) the total range of reaction to phenol was not bracketed: none of the concentrations of up to and including 100 ppm. suppressed growth. Strain 3 showed a lower growth rate than the other strains and this was the experience of essentially all of the experiments. However, little other significant difference in the growth of the strains was observed. In Experiment K., performed in the summer of 1966, concentrations of $CuSO_4$ from 0-10 ppm. permitted growth and no significant

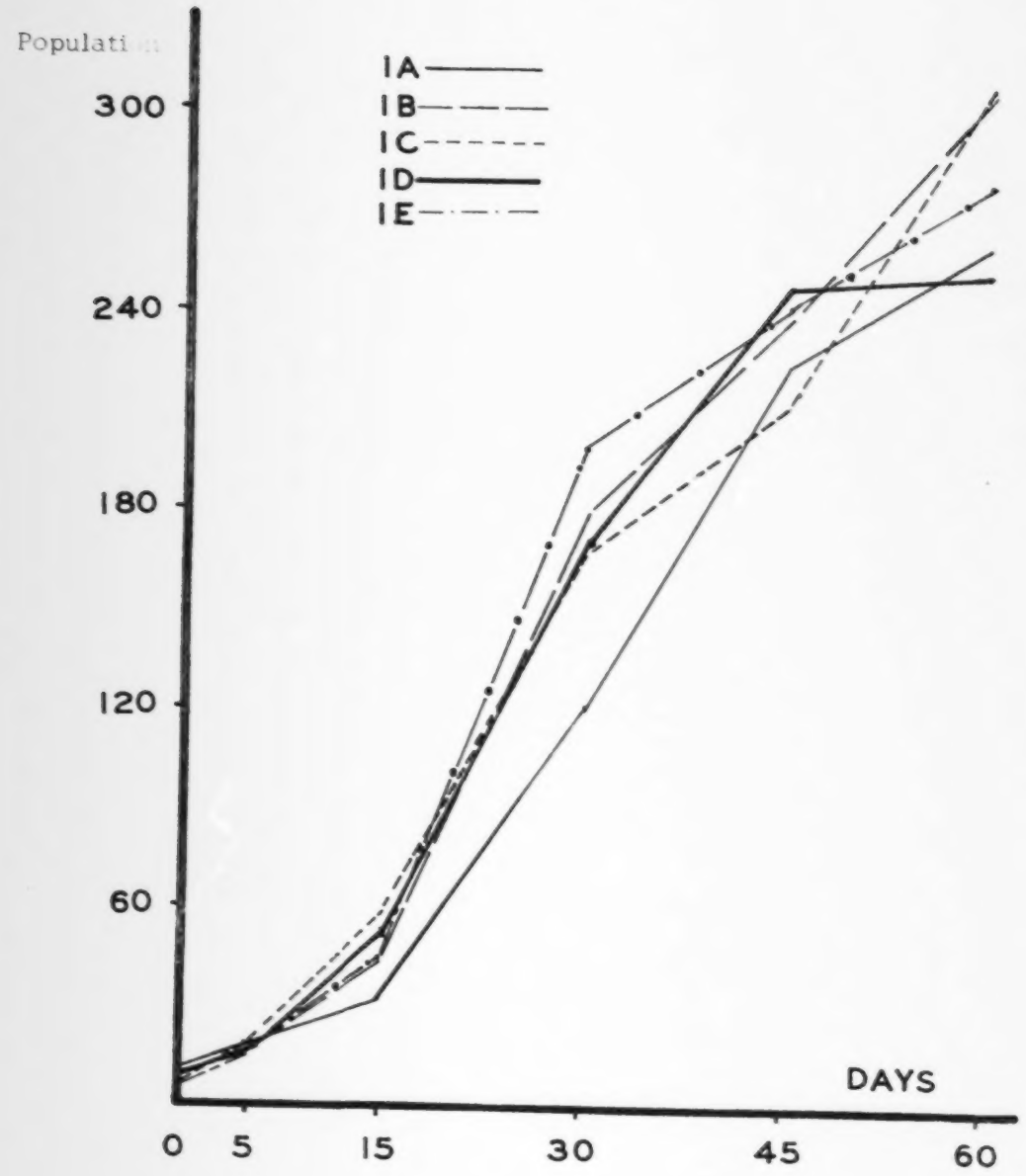

FIG. 9. Total population of coenobes/10^{-4}ml.

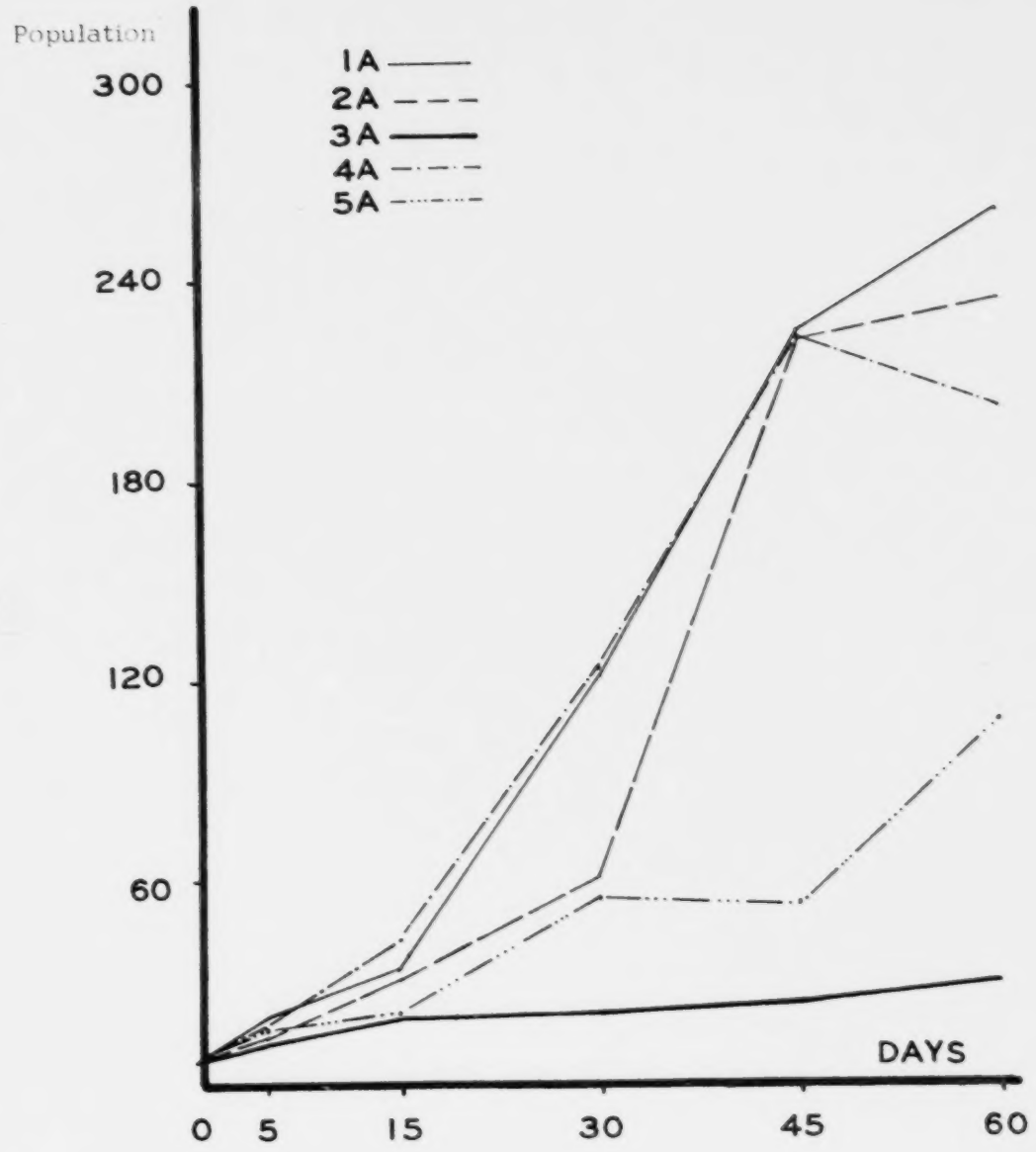

FIG. 10. Total population of coenobes/10^{-4}ml.

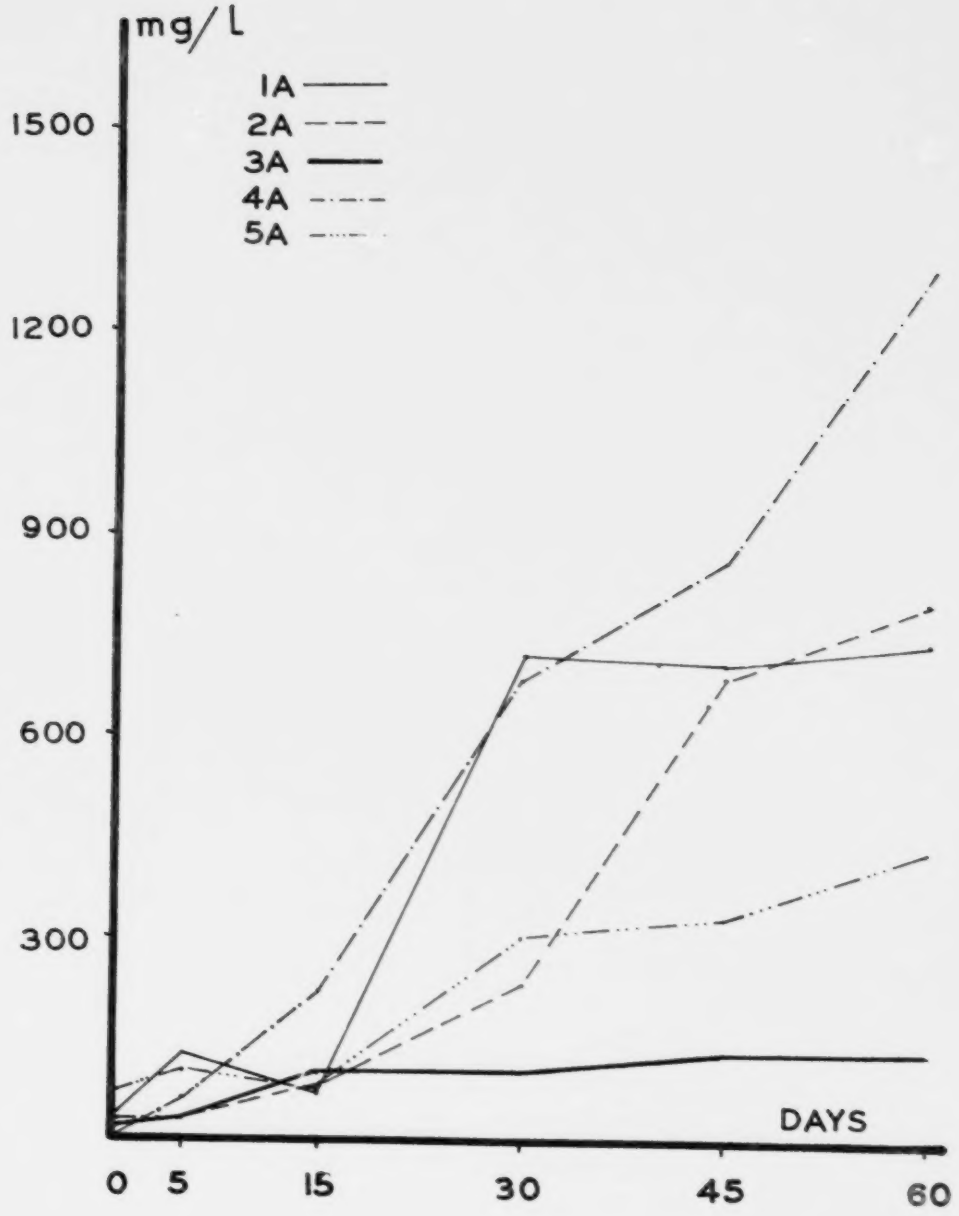

FIG. 11. Chlorophyll a content of cultures in mg/l.

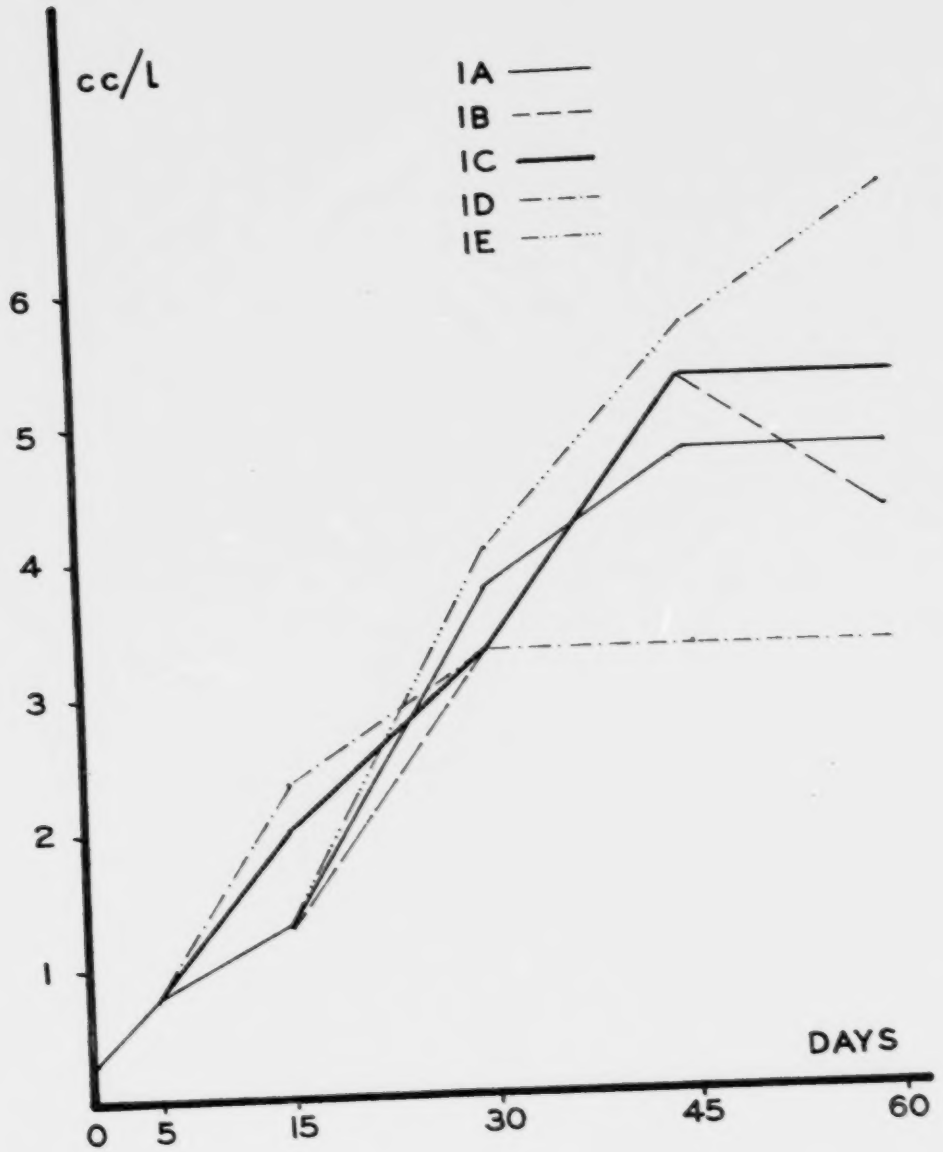

FIG. 12. Volume of population in cc/l.

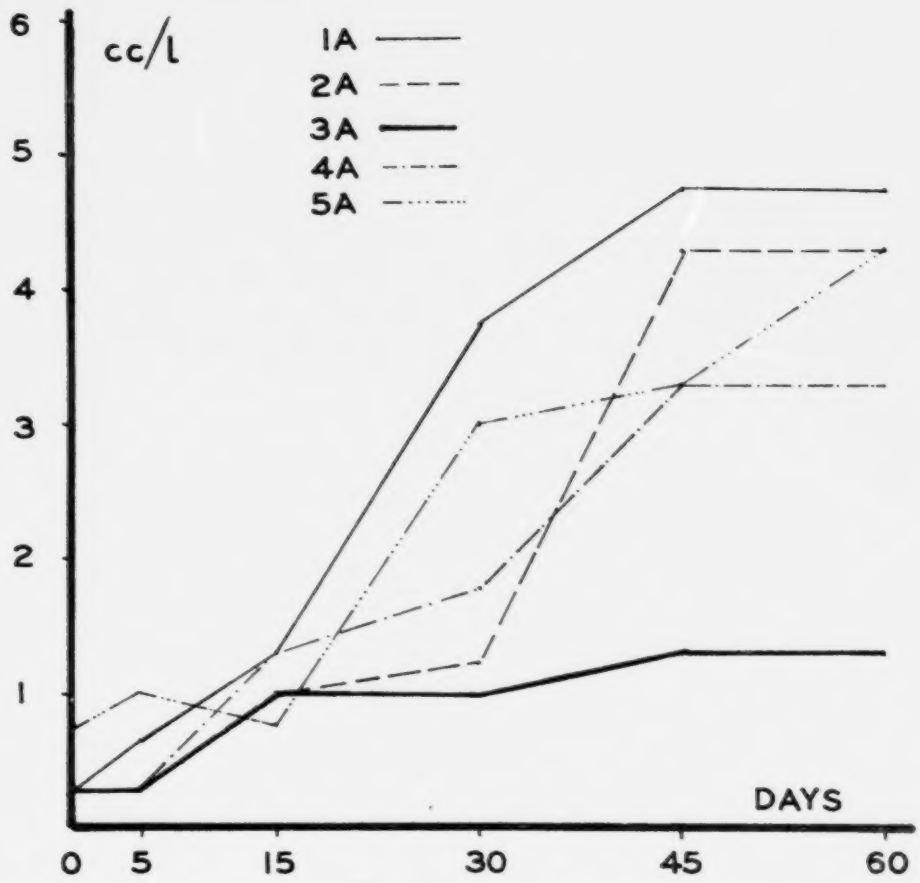

FIG. 13. Volume of population in cc/l.

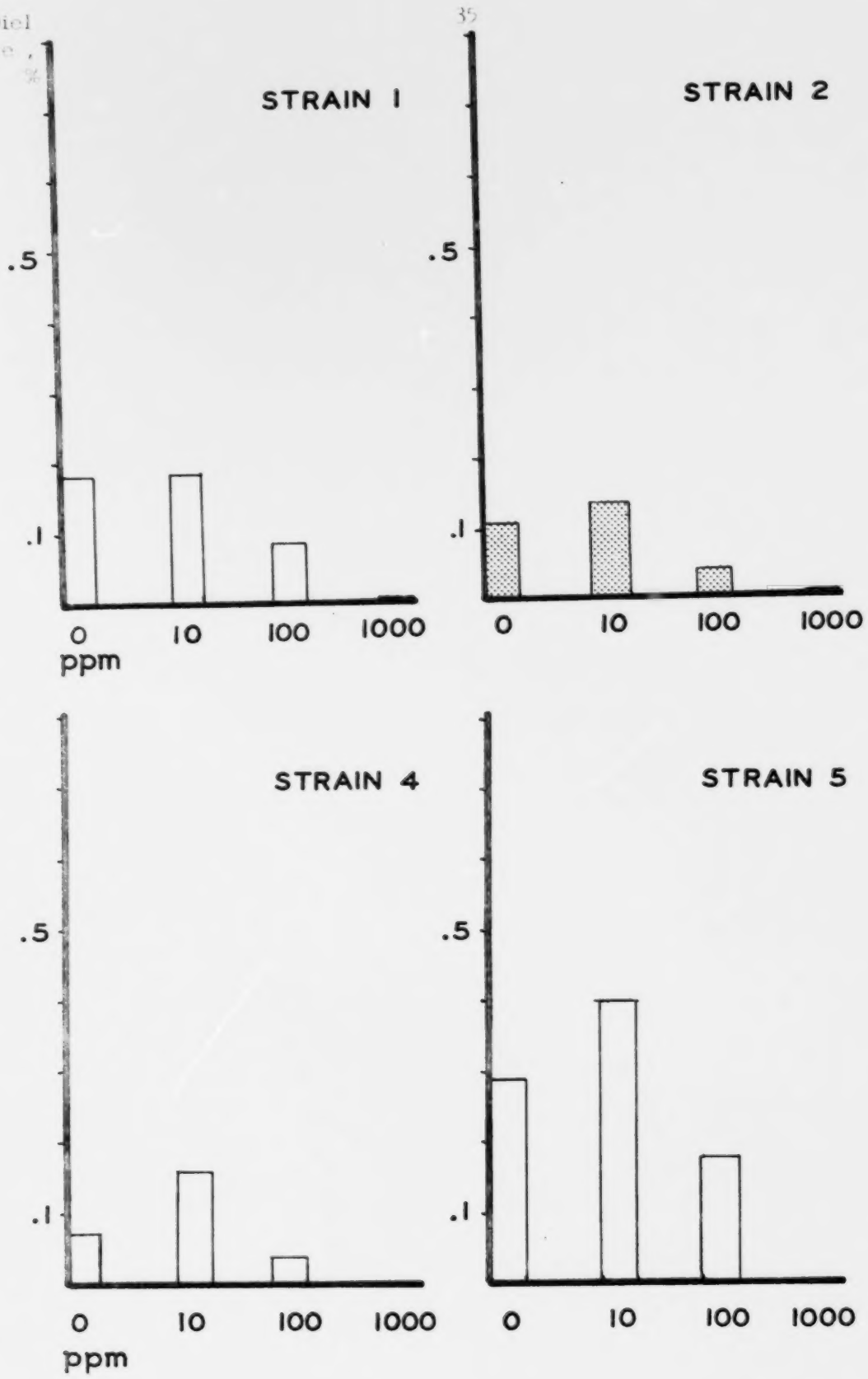

FIG. 14. Experiment M.
(Stippling represents contaminated cultures.)

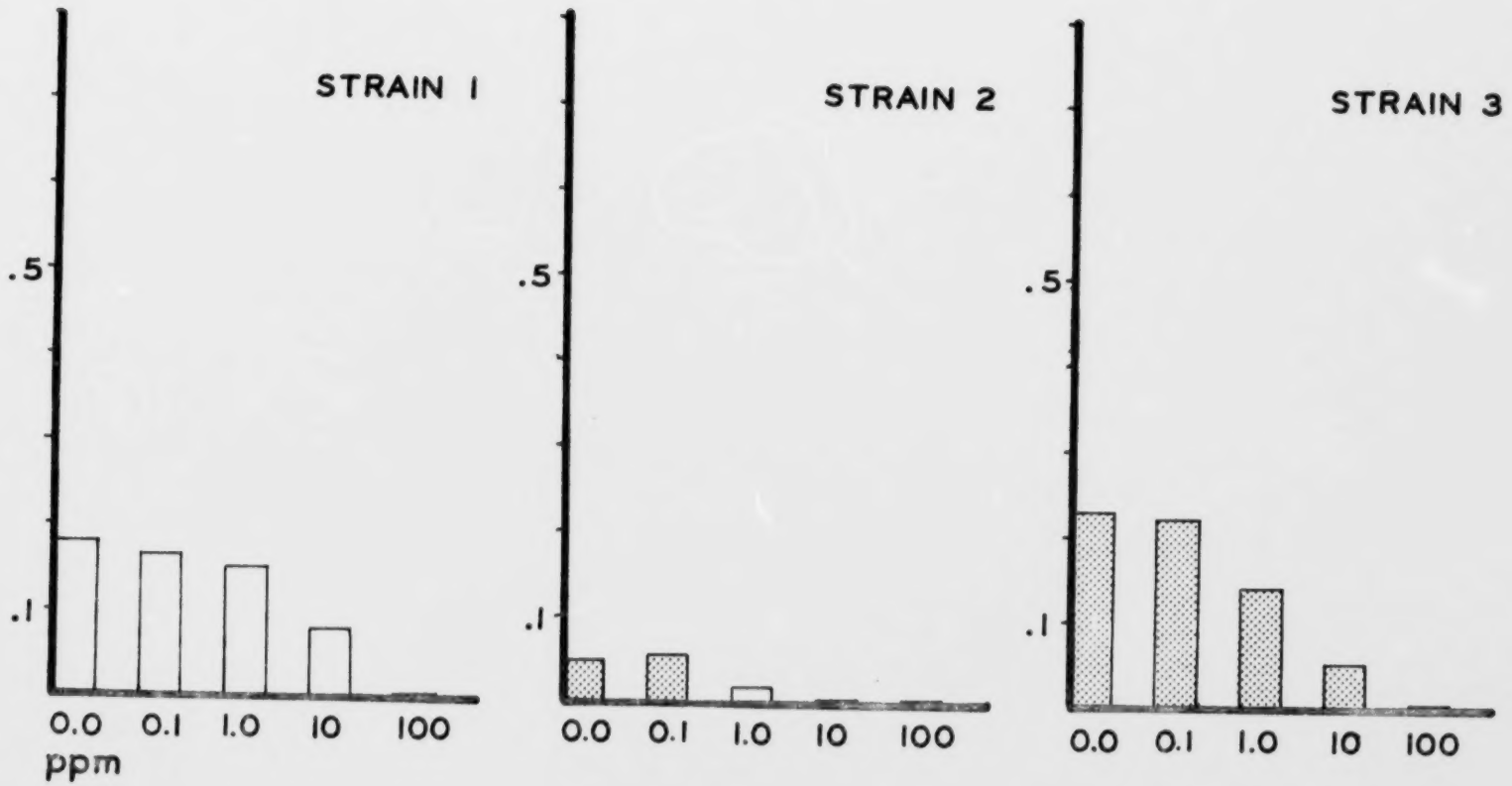

FIG. 15. Experiment N.

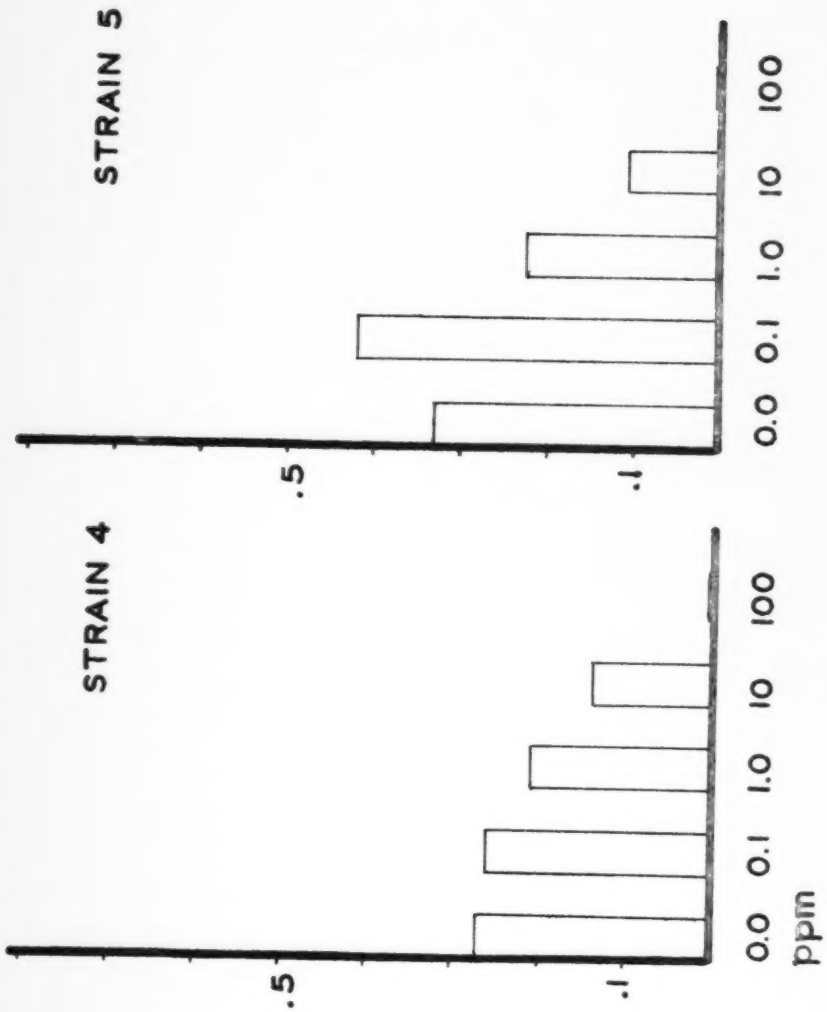

FIG. 16. Experiment N.

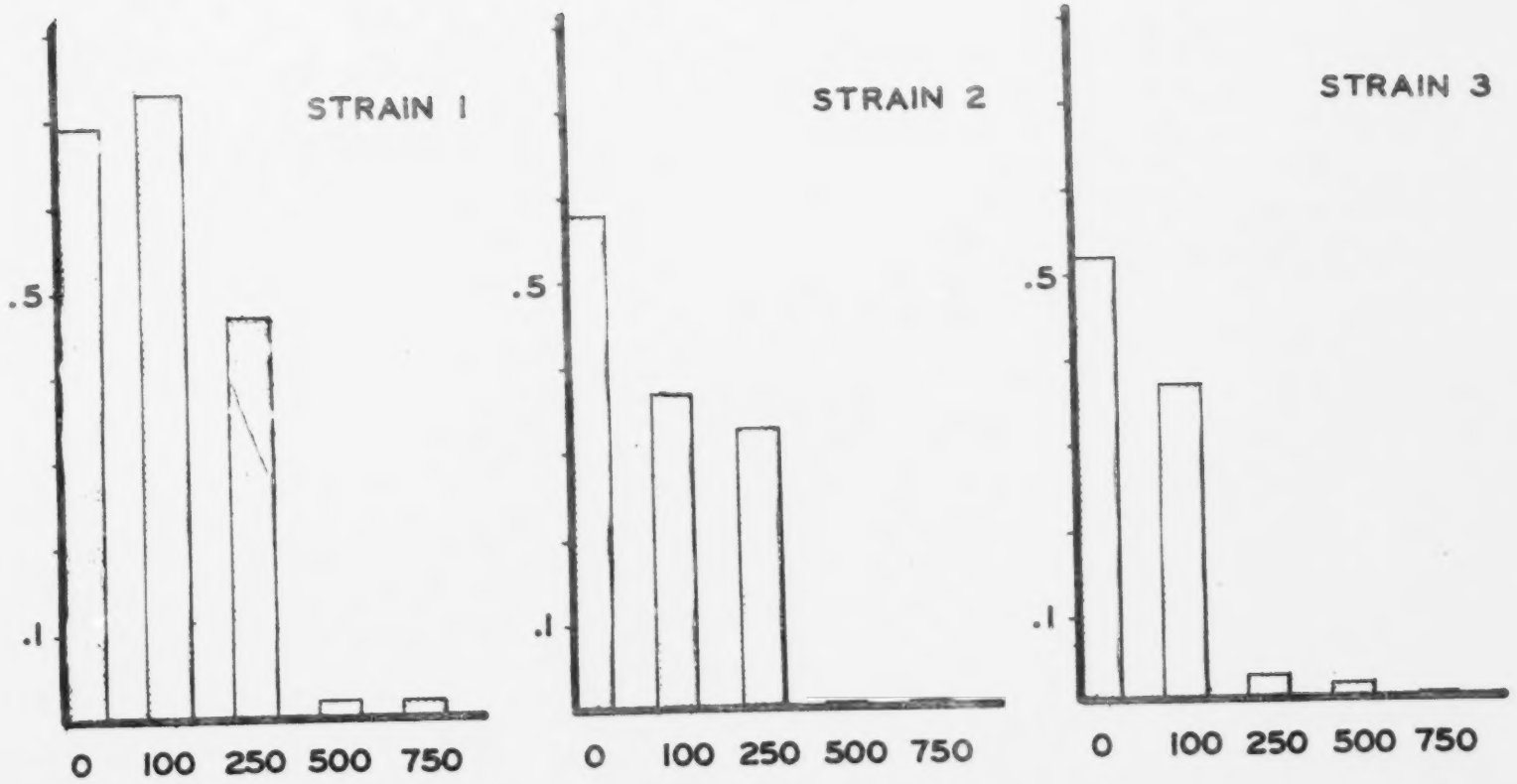

FIG. 17. Experiment O.

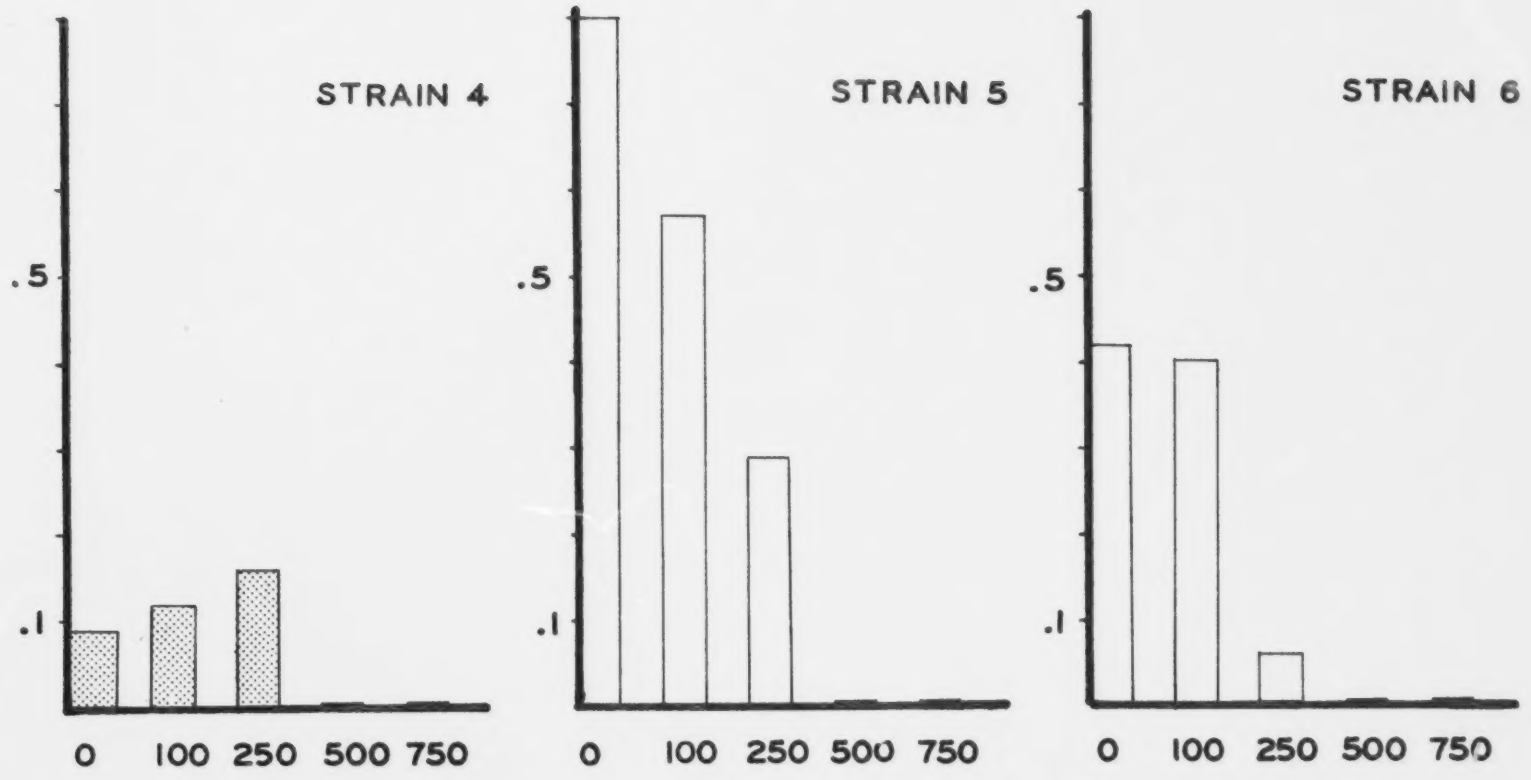

FIG. 18. Experiment O.

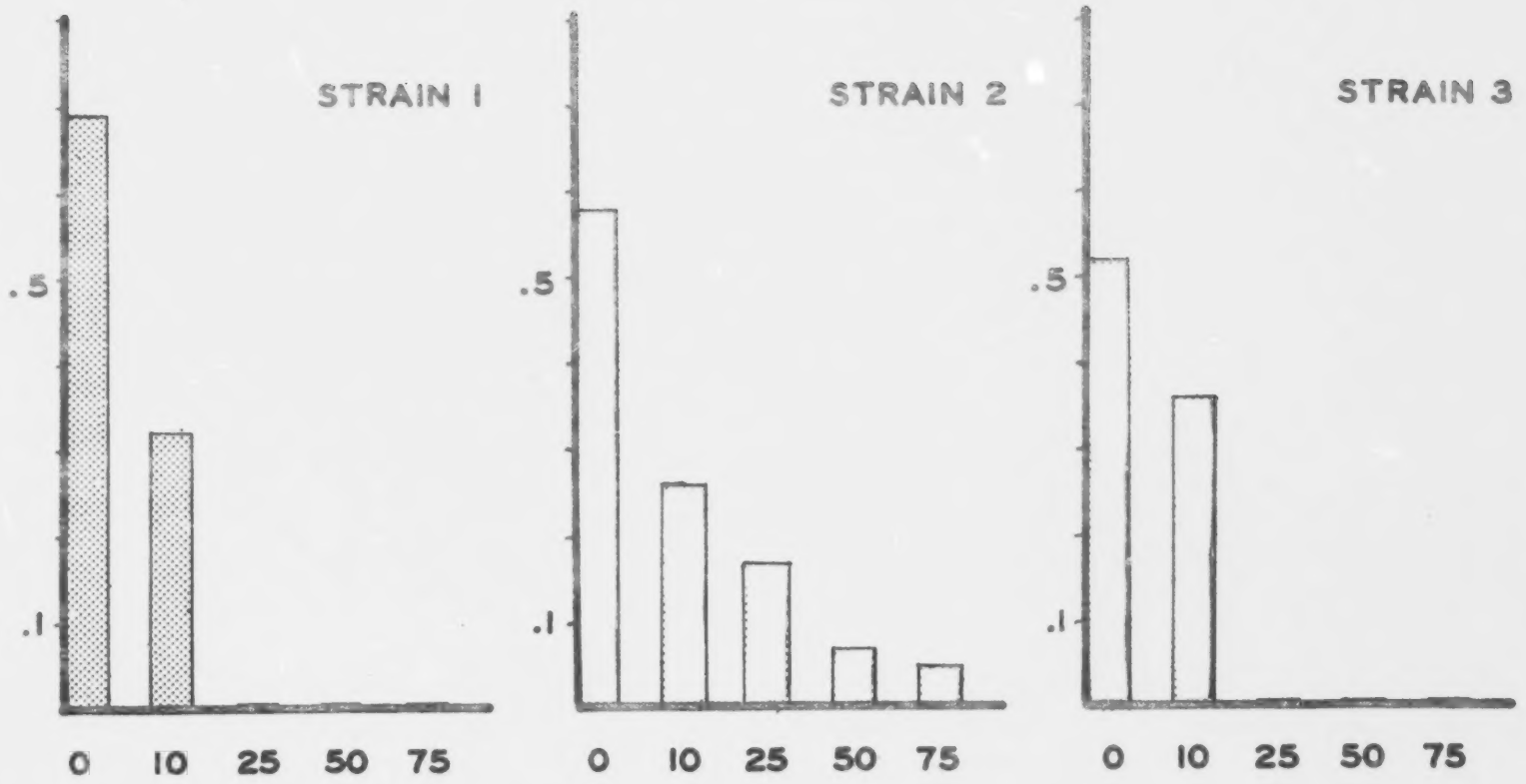

FIG. 19. Experiment P.

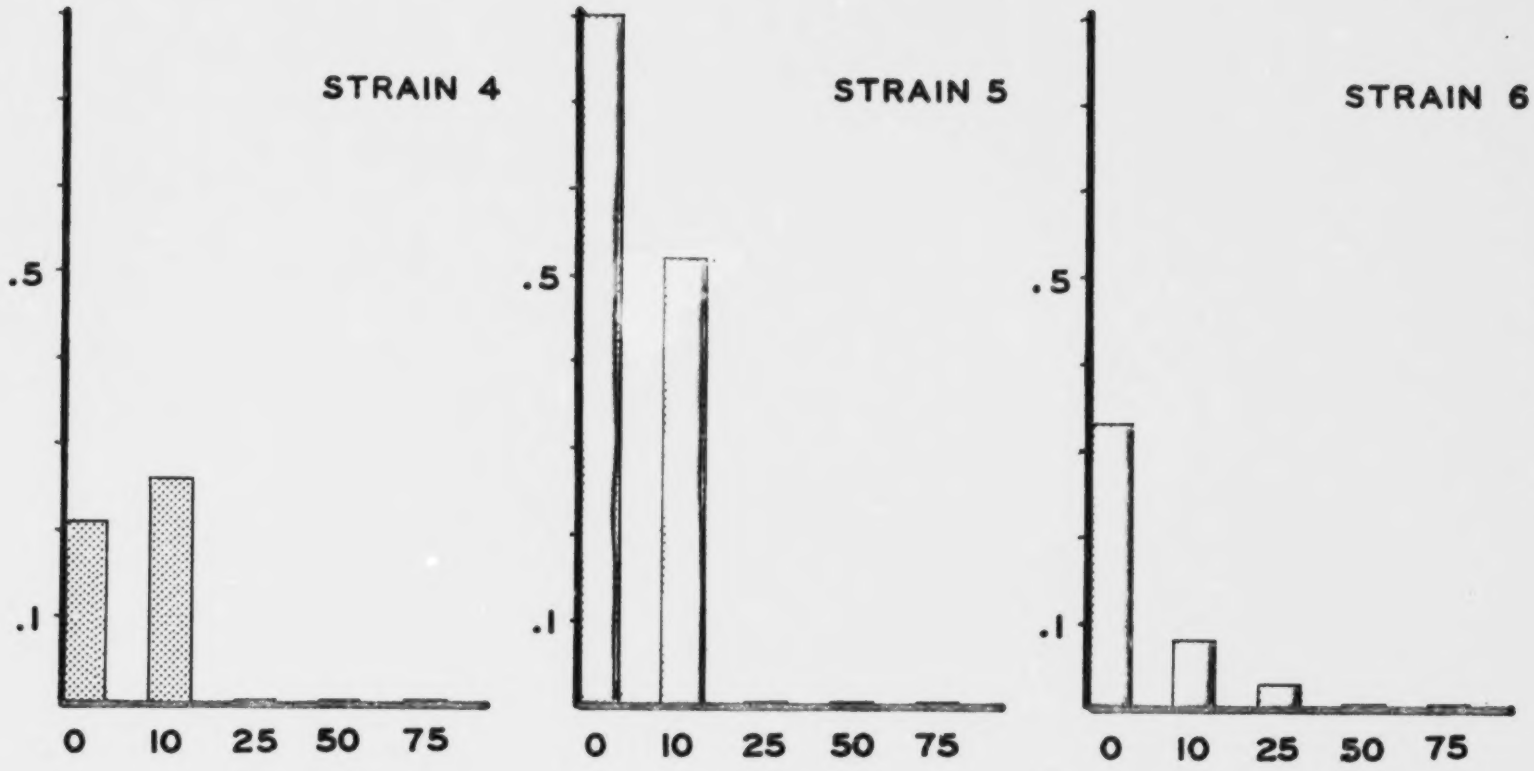

FIG. 20. Experiment P.

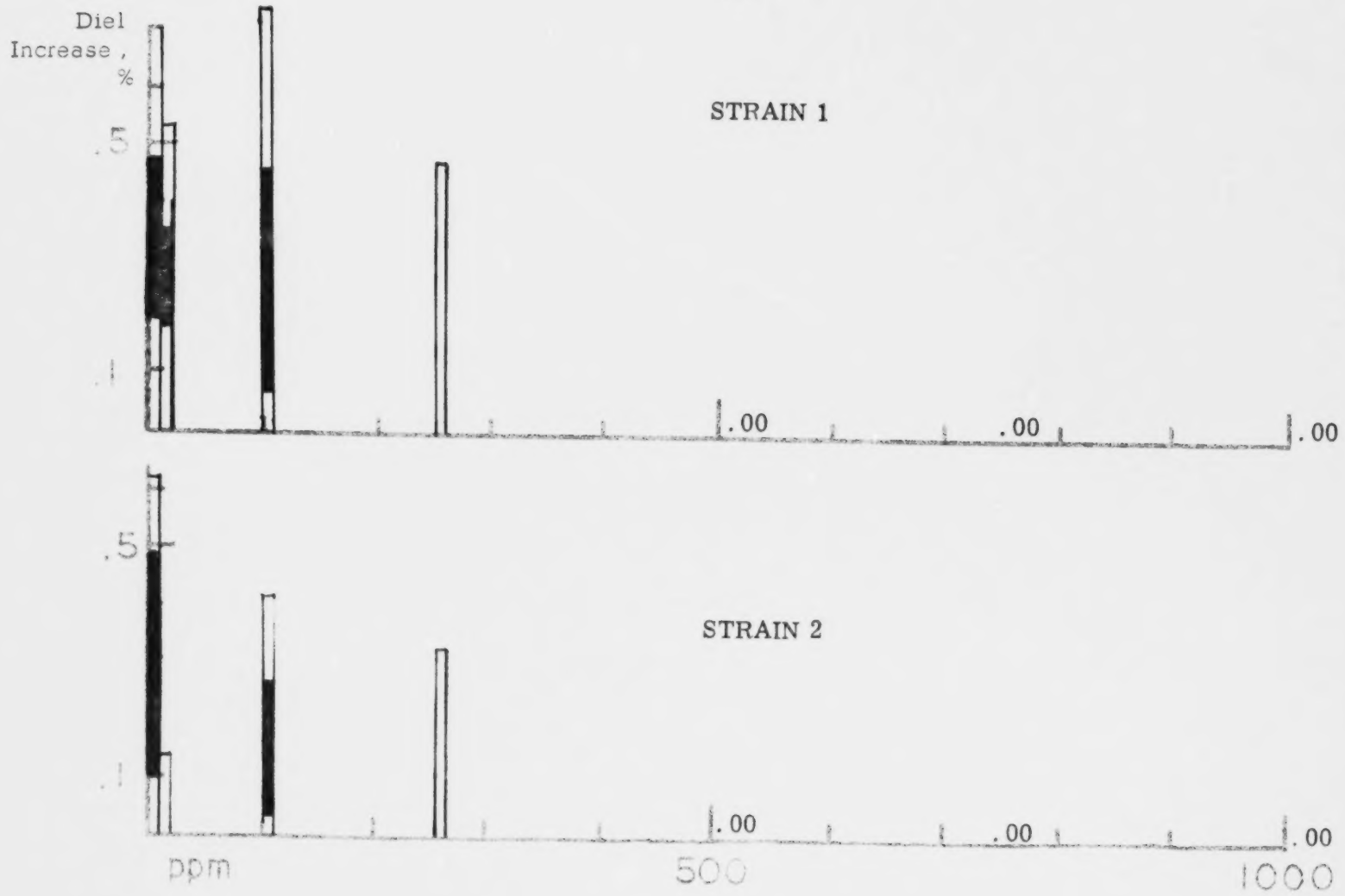

FIG. 21. Summary graph, phenol. Strains 1 and 2. In this and following figures (Figs. 22-26) the black bar provides a reference to mean and minimum readings: the upper end of the black bar represents the mean, the lower end of the black bar the minimum. The upper end of the white bar represents the maximum reading.

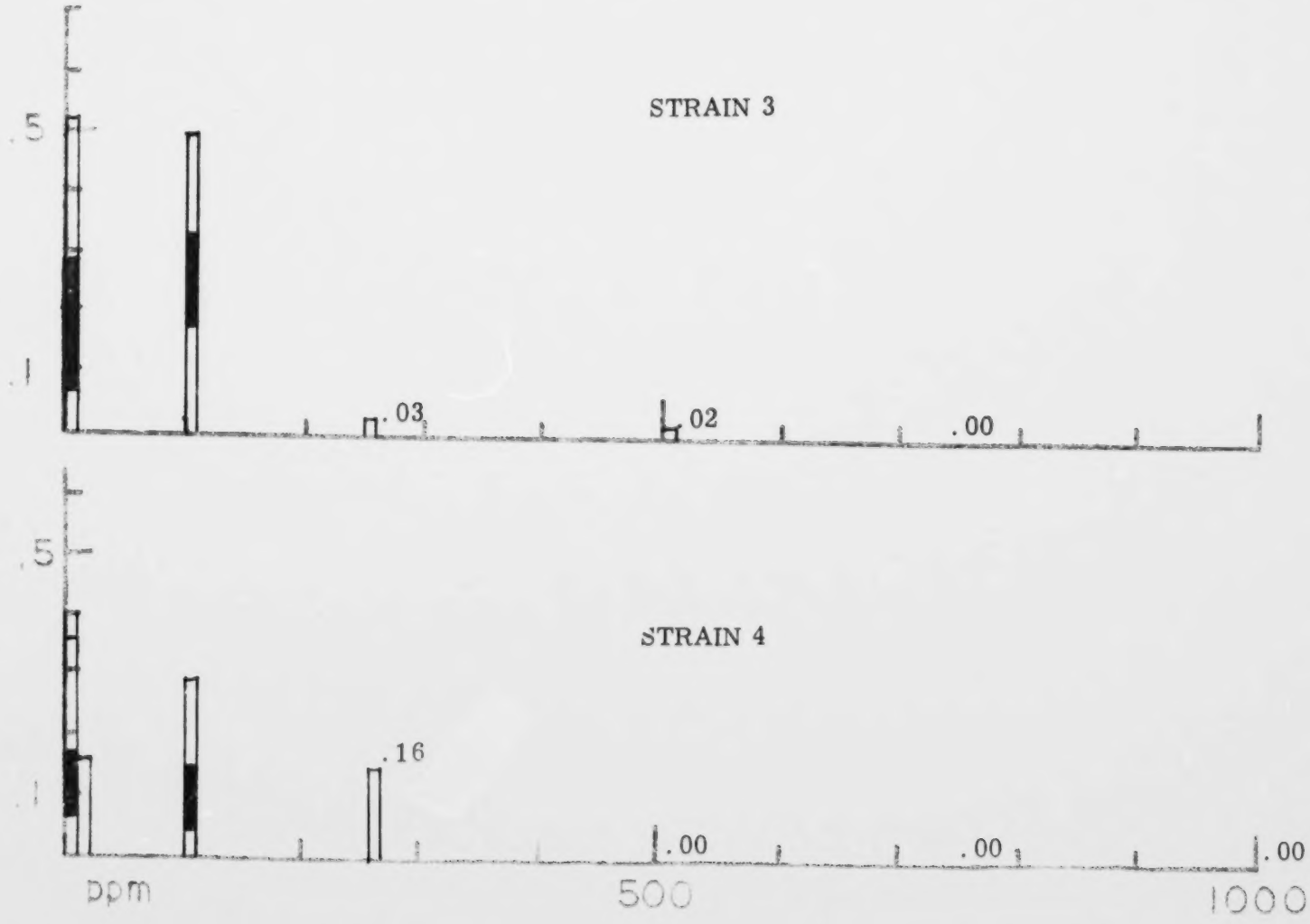

FIG. 22. Summary graph, phenol. Strains 3 and 4.

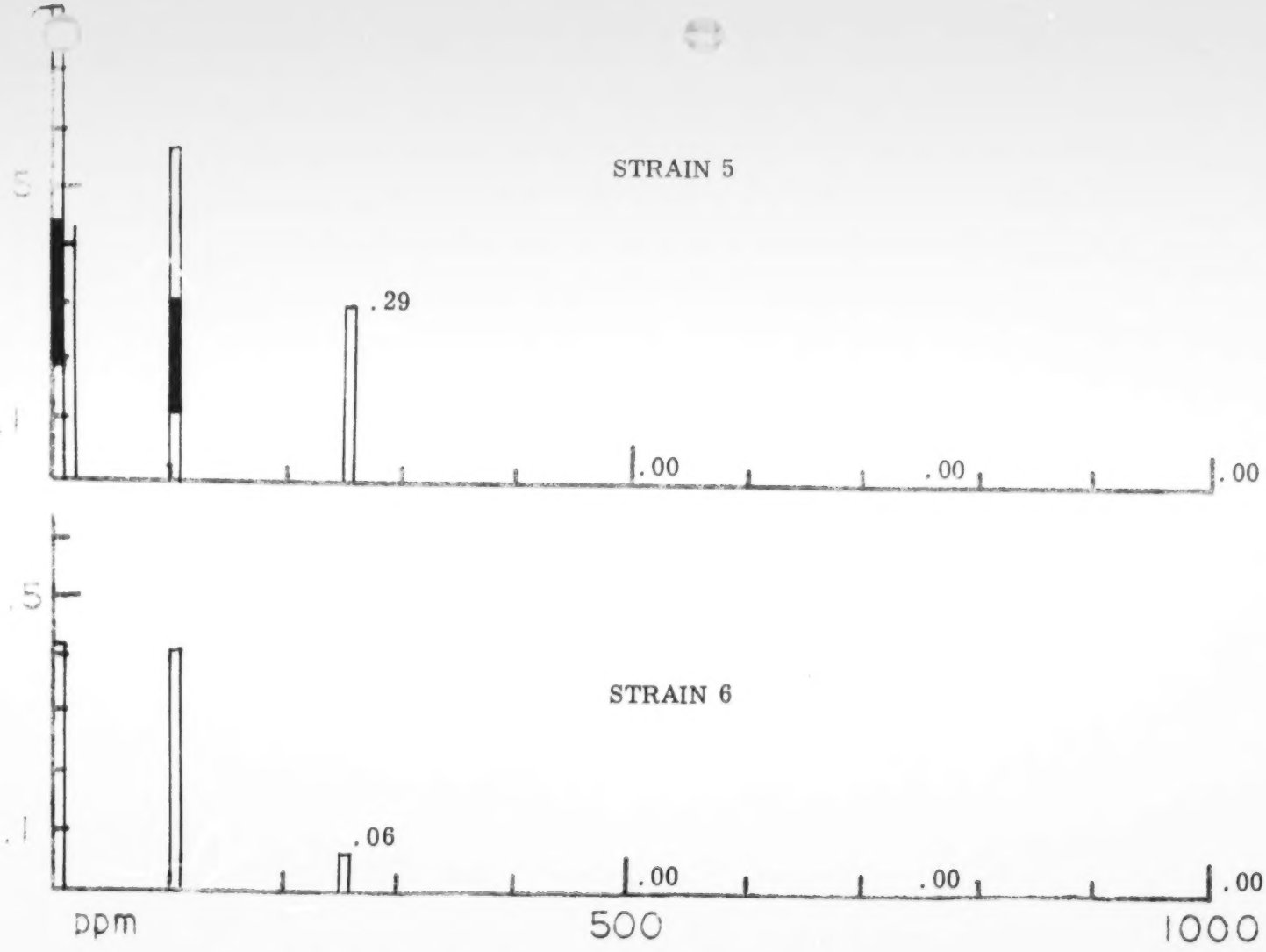

FIG. 23. Summary graph, phenol. Strains 5 and 6.

FIG. 24. Summary graph, $CuSO_4$. Strains 1 and 2.

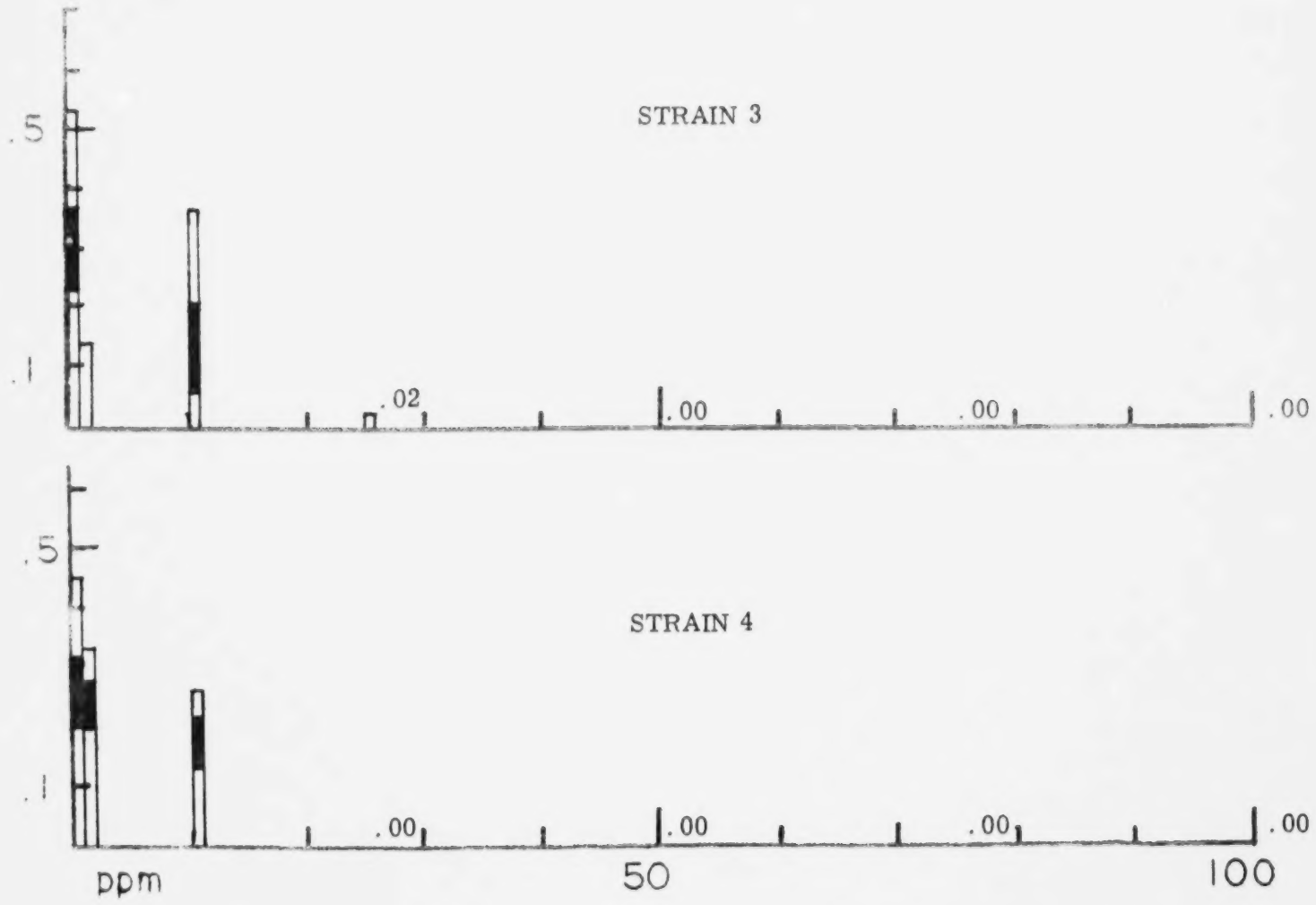

FIG. 25. Summary graph, $CuSO_4$. Strains 3 and 4.

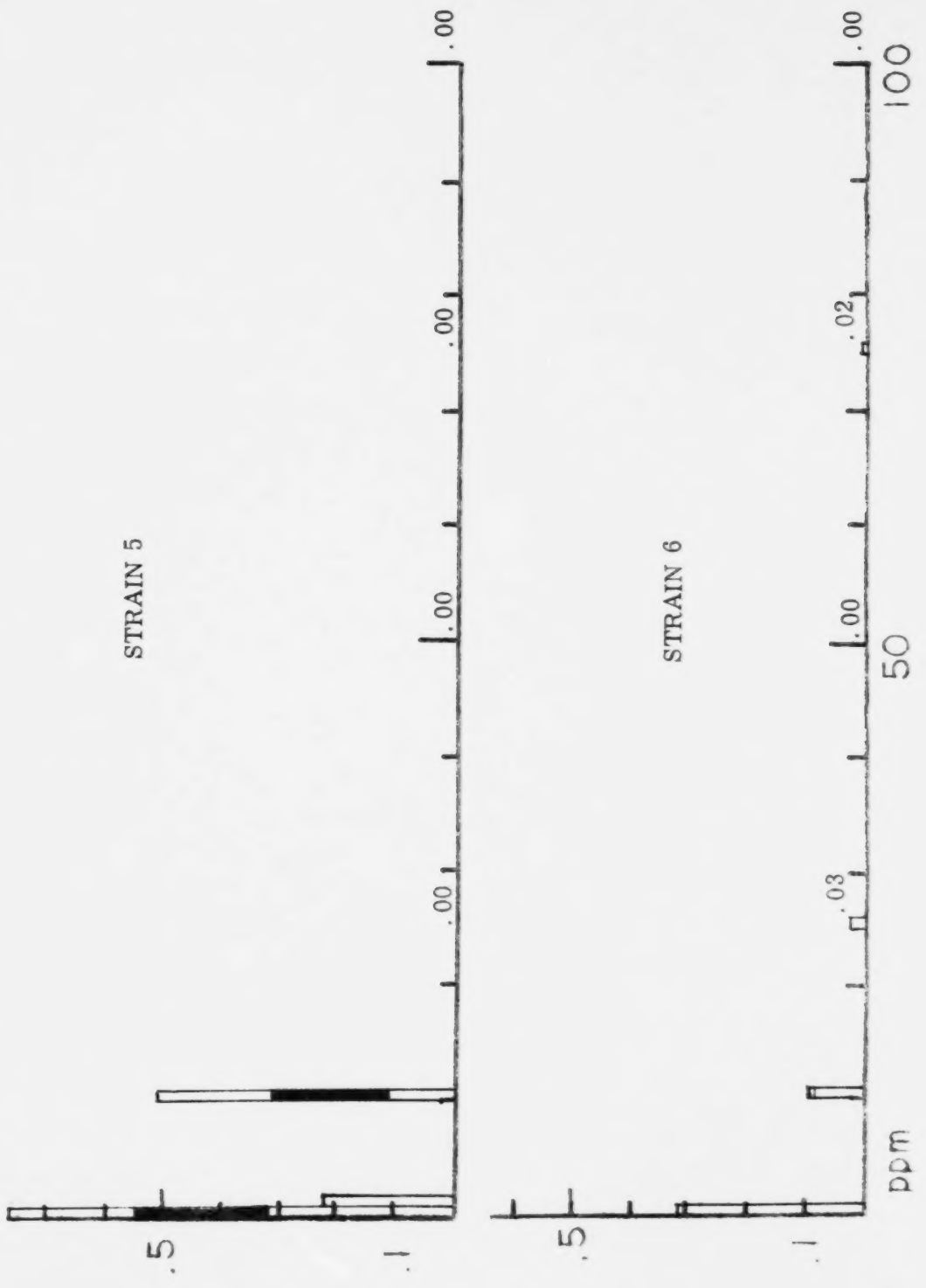

FIG. 26. Summary graph, $CuSO_4$. Strains 5 and 6.

difference was observed between concentrations (Table 11). Concentration E, initially 100 parts per million, was lethal. In Experiment L., there was little difference between any of the concentrations (of detergent) between 0-100 ppm. At the level of 1,000 ppm. growth ceased. In Experiment N., the concentration of phenol was increased to 1,000 ppm. which was clearly lethal for all strains investigated. Experiment M was essentially an effort to repeat, but without contamination, Experiment K with a larger number of strains. The initial level at which copper sulphate became lethal under conditions of this experiment was shown to be between 10 and 100 ppm. for all strains. Most of the strains in this experiment showed definitely reduced growth at the 10 ppm. level. Strain 2 showed little growth throughout this experiment--we have no adequate explanation for this.

In Experiments O and P, a series of more closely grouped concentrations was employed for both the principal pollutants between the known lethal and known non-lethal level. In Experiment O, which employed initial concentrations of 100, 250, 500, 750 ppm. phenol, growth was evidently possible at 250 initial ppm. for most of the strains; but strains 3 and 6 showed little growth at this level, and growth of all the strains at higher levels was nil or so slight as to be insignificant.

In Experiment P, in which a similar narrow range of concentrations of copper sulphate was employed between initial 10 ppm. and 75 ppm. levels, the reaction of essentially all of the strains was alike except for strains 2 and 6. Growth was good at both the zero and 10 ppm. level and nil above 10 ppm. In strain 2, however, there is evidence of growth at initial 25, 50, and 75 ppm. levels. Strain 6 also exhibited a small amount of growth at 25 ppm. Growth shown in Experiment P by strain 2 indicates the

possibility of a real difference in reaction to $CuSO_4$ between strain 2 and the others. Strain 2 is apparently capable of reproducing at initial levels which destroy or inhibit Scenedesmus of other strains. While up to the present time we have not repeated this experiment, strain 2 has exhibited the type of reaction to a pollutant which this series of experiments was intended to reveal (Fig. 9-26).

DISCUSSION

It was evident from the results of Experiment J that the Scenedesmus strains used were capable of growing in medium containing vastly greater initial phenol concentrations than amounts present in even such streams as are grossly phenol-polluted. This circumstance did not alter the experimental plan, but it did indicate that amounts of phenol commonly found in streams receiving various industrial liquors and cooling waters are unlikely to be critical per se for S. quadricauda. Subsequent experiments bore this out. Because of the high pollutant concentrations necessary to achieve reduction in growth, it is unrealistic to suppose that concentrations of either phenol or of $CuSO_4$ commonly present in "polluted" streams are of significance in determining the presence or absence of this species unless there exist in nature strains which are much more sensitive to the presence of these pollutants than the ones in these experiments. It is possible of course, that other media would provide sufficiently different conditions so as to modify completely the reaction pattern of the algal strains. No attempt has been made here to investigate the effects of varying factors of the experimental environment other than the pollutants being tested, although incidental changes from experiment to experiment were made in the interest of refining techniques or of eliminating contamination. Although many of the strains showed a reduced growth in the lowest concentration of pollutant used, respectively 0.1 ppm. of copper sulphate and of phenol, growth was frequently

very good in higher concentrations of the pollutant. With phenol as pollutant, only when a concentration of 250 ppm. was reached was there a clear reduction in rate of growth--a reduction evident in essentially all of the clones employed. This was the lowest concentration at which clear evidence of algistasis by the pollutant was obtained. In copper sulphate such algistasis was first apparent at 10 ppm. initial concentration; at 25 ppm. all of the clones showed either greatly reduced growth or no growth at all, the major exception being strain 2 which was the only strain exhibiting growth at this level. Growth in strain 2, however, took place in much higher concentrations as well, at 50 and at 75 ppm. Experiments O and P were principally instrumental in providing these results. While the results are in themselves interesting, the variability of the test organism in relation to Cu is not demonstrated conclusively, since the latter experiments have not been repeated.

The significant result of Experiment P provides positive evidence of the existence of at least one strain (#2) of S. quadricauda which is more resistant to a possible industrial pollutant--Cu--than are other strains. There is no reason to suppose, however, that the source stream for Strain 2, the relatively unpolluted Cedar Creek (Wisconsin) carries at any time exceptionally large amounts of Cu. Thus, the genetic variability in resistance which S. quadricauda exhibits does not appear in this instance at least to be environmentally related. It is difficult to see how it could be so related at Cu and Phenol levels which were found necessary to bring about algistatic conditions. Since no evidence was found that genetic variability is related to environmental needs or exigencies, to this extent the position of S. quadricauda as an organism indicative of pollution is not assailed. This alga exhibits such good growth under these experimental conditions in media both with and without pollutant additives, however,

that it would be an unlikely indicator of either Cu, phenol or biodegradable detergent. Assuming phenomonally high levels of phenol or of Cu in water, the experimental results indicate the possibility of explaining the presence of abundant S. quadricauda as being due to genetic variability and cast doubt on its validity as a possible "indicator" organism. Thus, both at ordinary and at unnaturally high concentrations of the pollutants tested, evidence is presented to weaken the concept of S. quadricauda as an indicator of polluted water.

SUMMARY

An investigation of the genetic variability of Scenedesmus quadricauda strains when growing in cultures containing different concentrations of toxic pollutants revealed that in Bristol's medium relatively high levels of pollutant concentration are required for an algistatic effect. One of the strains showed an ability to grow at high levels of $CuSO_4$, levels that resulted in algistasis or death of other strains. It was not possible to correlate the origin of the resistant strain with high levels of Cu in the environment. Both the growth of S. quadricauda at relatively high and relatively low concentrations of Cu, phenol and biodegradable detergent, and the demonstration of a strain more resistant than others, are considered as evidence to weaken any status S. quadricauda may have as an indicator of polluted water.

Acknowledgements:

The following assistants took part in this project:

William Woelkerling

Jose Villate

Carol DeDobay

Jayne Freiburger

Ronald Baumann

Jon Sperling

In addition, Marian Pierce contributed the great majority of the figures. The project could not have been completed without the aid of assistants Woelkerling and Villate, who planned and helped execute many of the experimental details and gave very abundantly of their time in the exacting work of making visual counts. The assistance of NSF Undergraduate Research Participation funds of a project under the direction of Dr. Jakob Loewenberg is gratefully acknowledged. These funds permitted the assistance of Mlles. DeDobay and Freiburger on the project. The assistance of various personnel of the UWM Center for Great Lakes Studies, including particularly Miss Pierce and Mr. Ratko Ristic is gratefully acknowledged.

BIBLIOGRAPHY

1. American Public Health Association. 1960. Standard Methods for the Examination of Water and Wastewaters. 11 ed. 626 pp.

2. Anderson, L. E. 1954. Hoyer's solution as a rapid permanent mounting medium for bryophytes. The Bryologist 57: 242-244.

3. Basavskaya, S. S. and E. N. Markarova. 1959. The effect of phosphorus on light and dark reactions of photosynthesis in Scenedesmus quadricauda. Figiol. Rastenii (Trans.) 6: 160-166.

4. Beck, W. M. 1954. Studies in stream pollution biology. I. A simplified ecological classification of organisms. Quar. Jour. Fla. Acad. Sci. 17: 211-227.

5. Bisalputra, T 1965. The origin of the pectic layer of the cell wall of Scenedesmus quadricauda. Can. Jour. Bot. 43: 1549-1552.

6. _____ and T. E. Weier. 1963. The cell wall of Scenedesmus quadricauda. Am. Jour. Bot. 50: 1011-1019.

7. _____. 1964. The pyrenoid of Scenedesmus quadricauda. Am. Jour. Bot. 51: 881-892.

8. Castelfranco, P. and T. Bisalputra. 1965. Inhibitory effect of amitrole on Scenedesmus quadricauda. Am. Jour. Bot. 52: 222-227.

9. Chantanachat, S. and H. C. Bold. 1962. Phycological studies. II. Some algae from arid soils. Univ. Texas Pub. 6218. 74 pp. Ills.

10. DeDobay, Carol. 1966. National Science Foundation Undergraduate Research Project. 30 pp., typewritten.

11. Fitzgerald, G. P. and S. L. Faust. 1965. Effect of bacteria on the solubility of copper algicides. Water and Sewage Works Jour. 112: 271-275. 5 pp.

12. Fjerdingstad, E. 1964. Pollution of streams estimated by benthal phytomicro-organisms. I. A. saprobic system based on communities of organisms and ecological factors. Int. Revue ges. Hydrobiol. 49: 63-131.

13. Freiburger, Jayne. 1966. National Science Foundation Undergraduate Research Project. 50 pp., typewritten.

14. Gistl, R. 1932. Zur Kenntnis der Erdalgen. Bot. Inst. der Tech. Hochschule München. pp. 634-649.

15. Johansen, D. A. 1940. Plant Microtechnique. xi + 532 pp. New York.

15. Kolkwitz, R. and M. Marsson. 1908. Ökologie der pflanzlichen Saprobien. Ber. Deutsch. Bot. Ges. 26a: 505-519.

16. Kutkuhn, J. 1958. Notes on the precision of numerical and volumetric plankton estimates from small sample concentrates. Limn. and Oceanogr. 3: 69-83.

17. Liebmann, H. 1951. Handbuch der Frischwasser- und Abwasserbiologie I. 539 pp. München.

18. Lund, J. W. G., C. Kipling, and E. D. La Cren. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11: 143-170.

19. Osterlind, S. 1949. Growth conditions of the alga Scenedesmus quadricauda with special reference to the inorganic carbon sources. Symbol. Bot. Upsal. 10(3): 1-141.

20. Pratt, R. 1940. Influence of the size of the inoculum on the growth of Chlorella vulgaris in freshly prepared culture medium. Am. Jour. Bot. 27: 52-56.

21. Richards, F. A. and T. G. Thompson. 1952. The estimation and characterization of plankton populations by pigment analysis. II. A spectrophotometric method for the estimation of plankton pigments. Jour. Mar. Res. 11: 156-172.

22. Rodhe, W. 1948. Environmental requirements of fresh-water plankton algae. Symb. Bot. Upsal. 10(1): 1-149.

23. Simpson, G. G., A. Roe, and R. C. Lewontin. 1960. Quantitative Zoology. vii + 440 pp. New York.

24. Sorensen, I. 1950. Über biologische Reinigung phenolhaltiger Abwässer. Kungl. Fysiog. Sallsk. und Forhand. 20: 1-10.

25. Šrámek-Hušek, R. 1956. Zur biologischen Charakteristik der höheren Saprobitätsstufen. Arch. Hydrobiol. 51: 376-390 (not seen).

26. Swale, E. 1967. Scenedesmus with Chodatella-stages. Br. Phyc Bull. 3: 281--293.

27. Trainor, F. R. 1963. The morphology of Scenedesmus in pure and contaminated culture. Bull. Torrey Bot. Club 90: 137-138.

28. _____ 1964. Spine distribution in several Scenedesmus cultures. Am. Jour. Bot. 51: 995-1001.

29. _____ 1965. A study of unialgal cultures of Scenedesmus incubated in nature and in the laboratory. Can. Jour. Bot. 43: 701-706.

30. _____ 1966. A study of wall ornamentation in cultures of Scenedesmus. Am. Jour. Bot. 53: 995-1,000.

31. _____ and C. A Burg. 1965. Motility in Scenedesmus dimorphus, Scenedesmus obliquus, and Coelastrum microporum. Jour. Phycol. 1: 14-17.

32. Winokur, Morris. 1948. Growth relationships of Chlorella spp. Am. Jour. Bot. 35: 118-129.

33. Woelkerling, W. 1966. The effects of genetics and environment on the ecology of algal indicator organisms. M. S. Thesis, Univ. of Wisc.-Milwaukee. 1 + 69 pp.

APPENDIX

(Figure 27 and Tables 1-19).

Haemacytometer Tally Sheet

STRAIN _____ TEST DAY _____

DILUTION FACTOR _____ OBSERVER _____

Remarks:

Count	Total	2-celled	4-celled	8-celled
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
Grand Totals				
Grand Averages				
Averages				
Corrected Averages (Includes D. F.)				

Grand Average = (Grand Total)/No. of counts

Average = (Grand Average)/9, to obtain organisms/0.1 cubic mm.

Corrected Average = (Average) x (D. F.)

To obtain organisms/ml, multiply by 10,000

Figure 27

TABLE 1

Experiment J--Summary

Pollutant: Phenol
Duration: 60 days
Start: 1-1-66. Termination: ca. 3-1-66.

Test days: 0, 5, 15, 30, 45, 60.
Cultures used: 1, 2, 3, 4, 5, 6.
Contaminated before end of experiment: 3, some of 5.
Concentration code:
A = 0.0 ppm
B = 0.1 ppm
C = 1.0 ppm
D = 10.0 ppm
E = 100 ppm
In charge: Blum, Woelkerling
Inoculation: Woelkerling, Villate
Counts: Woelkerling, Villate

Strain	Cell conc./4 zero day	0 + 60	Increase factor	Increase factor/60
1A	73,600	2,241,000	30.44	.507
1B	"	2,691,000	36.56	.609
1C	"	2,580,500	35.06	.601
1D	"	378,400	32.44	.540
1E	"	2,477,500	33.66	.561
2A	42,000	1,933,500	46.03	.767
2C	"	1,378,500	32.82	.547
2E	"	1,060,500	25.25	.420
3A	38,000	158,500	4.17	.069
3C	"	152,500	4.01	.066
3E	"	386,500	10.17	.169
4A	84,100	1,841,000	21.89	.364
4C	"	1,711,000	20.34	.339
4E	"	1,546,000	18.38	.306
5A	82,600	966,500	11.70	.195
5C	"	738,000	8.93	.148
5E	"	549,000	6.64	.110

TABLE 2

Experiment K--Summary

Pollutant: $CuSO_4$
Duration: 28 days
Start: 6-15-66
Terminated: 7-13-66
Test days: 0, 28.
Cultures used: 1, 2, 4.
Contaminated before end of experiment: 1, 4.
Concentration code:
A = 0 ppm
B = 0.1 ppm
C = 1.0 ppm
D = 10.0 ppm
E = 100 ppm
In charge: Woelkerling, Freiburger
Inoculation: Woelkerling, Freiburger, Villate
Counts: Freiburger, Villate, Woelkerling, Blum

Strain	Cell conc./4 zero day	0 + 28	Increase factor	Increase factor/28
1A	97,500	1,092,000	11.20	0.40
1B	108,000	725,500	6.71	0.23
1C	103,000	940,500	9.13	0.32
1D	98,000	1,027,000	10.47	0.37
1E	124,000	----	----	----
2A	59,500	159,500	2.67	0.09
2B	92,000	160,000	1.73	0.06
2C	92,000	271,500	2.95	0.10
2D	91,000	350,500	3.85	0.13
2E	78,000	----	----	----
4A	72,000	887,500	12.63	0.45
4B	101,000	1,564,000	15.48	0.55
4C	130,000	1,263,000	9.71	0.34
4D	135,000	1,001,500	7.41	0.26
4E	122,000	----	----	----

TABLE 3

Experiment L--Summary

Pollutant: Biodegradable detergent
Duration: 28 days
Start: 6-15-66
Terminated: 7-13-66
Test days: 0, 28.
Cultures used: 1, 2, 4.
Contaminated before of experiment: 2.
Concentration code:
A = 0 ppm
B = 1 ppm
C = 10 ppm
D = 100 ppm
E = 1000 ppm
In charge: Woelkerling, DeDobay
Inoculation: Woelkerling, DeDobay, Villate
Counts: DeDobay, Villate, Woelkerling, Blum

Strain	Cell conc./4 zero day	0 + 28	Increase factor	Increase factor/60
1A	48,000	193,000	4.02	0.06
1B	40,000	274,000	6.85	0.11
1C	42,000	180,000	4.28	0.07
1D	40,000	161,000	4.02	0.06
1E	46,000	----	----	----
2A	64,000	489,000	7.64	0.12
2B	61,000	1,509,000	24.73	0.41
2C	63,000	671,000	10.65	0.17
2D	62,000	1,181,000	19.04	0.31
2E	46,000	0	0	0
3A	72,000	504,000	7.00	0.11
3B	65,000	257,000	3.95	0.06
3C	72,000	333,000	4.62	0.07
3D	65,000	281,000	4.32	0.07
3E	66,000	----	----	----

TABLE 4

Experiment M--Summary

Pollutant: Phenol
Duration: 46 days
Start: 7-27-67
Terminated: 9-11-67
Test days: 0, 46.
Cultures used: 1, 2, 3, 4, 5.
Contaminated before end of experiment: 2, 3.
Concentration code:
A = 0.0 ppm
B = 10 ppm
C = 100 ppm
D = 1000 ppm
In charge: Blum, Villate
Inoculation: Villate, Blum
Counts: Villate, Baumann, Sperling, Blum

Strain	Cell conc./4 zero day	0 + 46	Increase factor	Increase factor/46
1A	217,300	1,834,400	8.44	.183
1B	"	1,857,900	8.54	.185
1C	"	846,900	3.89	.084
1D	"	95,500	.43	----
2A	320,400	1,613,300	5.03	.109
2B	"	2,021,900	6.31	.137
2C	"	634,100	1.97	.042
2D	"	22,800	0.07	----
4A	273,300	884,900	3.23	.070
4B	"	2,027,000	7.41	.161
4C	"	570,300	2.08	.045
4D	"	31,000	----	----
5A	24,200	333,300	13.77	.299
5B	"	449,000	18.55	.403
5C	"	211,300	8.73	.189
5D	"	9,300	0.38	----

TABLE 5

Experiment N--Summary

Pollutant: $CuSO_4$
Duration: 46 days
Start: 7-27-67
Terminated: 9-11-67
Test days: 0, 46.
Cultures used: 1, 2, 3, 4, 5.
Contaminated before end of experiment: 3.
Concentration code:
A = 0.0 ppm
B = 0.1 ppm
C = 1 ppm
D = 10 ppm
E = 100 ppm
In charge: Blum, Villate
Inoculation: Villate, Blum
Counts: Villate, Baumann, Blum, Sperling

Strain	Cell conc./4 zero day	0 + 46	Increase factor	Increase factor/46
1A	217,300	1,922,700	8.84	.192
1B	"	1,655,300	7.61	.165
1C	"	1,501,200	6.90	.150
1D	"	832,350	3.83	.083
1E	"	85,770	----	----
2A	320,400	785,900	2.45	.053
2B	"	935,200	2.91	.063
2C	"	429,400	1.34	.029
2D	"	100,000	0.31	.006
2E	"	50,000	----	----
3A	148,200	1,554,000	10.48	.227
3B	"	1,514,700	10.22	.222
3C	"	982,700	6.63	.144
3D	"	382,700	2.58	.056
3E	"	22,000	----	----
(Strain 4 not visually counted.)				
4A	50,000	630,000	12.60	.273
4B	50,000	600,000	12.00	.260
4C	50,000	500,000	10.00	.217
4D	50,000	320,000	6.40	.139
4E	50,000	50,000	----	----
5A	24,200	370,700	15.31	.332
5B	"	476,600	19.69	.428
5C	"	253,500	10.47	.227
5D	"	129,500	5.35	.116
5E	"	10,600	----	----

TABLE 6

Experiment O--Summary

Pollutant: Phenol
Duration: 48 days
Start: 12-24-67
Terminated: 9-11-68
Test days: 0, 48.
Cultures used: 1, 2, 3, 4, 5, 6.
Contaminated before end of experiment: 4, 5.
Concentration code:
A = 0.0 ppm
B = 100 ppm
C = 250 ppm
D = 500 ppm
E = 750 ppm
In charge: Blum, Villate
Inoculation: Villate, Blum
Counts: Villate, Baumann, Blum, Sperling

Strain	Cell conc./4 zero day	0 + 48	Increase factor	Increase factor/48
1A	93,000	3,123,100	33.58	0.699
1B	"	3,229,400	35.47	0.738
1C	"	2,126,000	22.86	0.476
1D	"	107,200	1.15	.023
1E	"	104,500	1.12	.023
2A	119,500	3,353,700	28.06	.584
2B	"	2,134,400	17.86	.372
2C	"	1,934,700	16.18	.337
2D	"	99,600	----	----
2E	"	110,400	----	----
3A	39,700	1,008,300	25.39	.528
3B	"	955,500	24.06	.501
3C	"	64,100	1.61	.033
3D	"	42,600	1.07	.022
3E	"	37,100	----	----
4A	118,500	527,500	4.45	.092
4B	"	713,300	6.01	.125
4C	"	906,700	7.65	.159
4D	"	104,400	----	----
4E	"	87,900	----	----
5A	32,300	1,251,600	38.74	.807
5B	"	888,500	27.50	.572
5C	"	451,100	13.96	.290
5D	"	27,500	----	----
5E	"	29,100	----	----
6A	30,200	616,400	20.41	.425
6B	"	584,900	19.36	.403
6C	"	93,700	3.10	.064
6D	"	29,100	----	----
6E	"	28,700	----	----

TABLE 7

Experiment P--Summary

Pollutant: $CuSO_4$
Duration: 48 days
Start: 12-24-67
Terminated: 2-10-68
Test days: 0, 48.
Cultures used: 1, 2, 3, 4, 5, 6.
Contaminated before end of experiment: 4, 5.
Concentration code:
A = 0.0 ppm
B = 10 ppm
C = 25 ppm
D = 50 ppm
E = 75 ppm
In charge: Blum, Villate
Inoculation: Villate, Blum
Counts: Villate, Baumann, Blum

Strain	Cell conc./4 zero day	0 + 48	Increase factor	Increase factor/48
1A	93,000	3,123,200	33.58	0.699
1B	"	1,448,900	15.57	0.324
1C	"	95,700	1.02	.021
1D	"	104,100	1.26	.026
1E	"	91,800	----	----
2A	119,500	3,353,800	28.06	.584
2B	"	1,495,800	12.51	.260
2C	"	1,016,500	8.50	.177
2D	"	443,300	3.70	.077
2E	"	305,200	2.55	.053
3A	39,700	1,008,300	25.39	0.528
3B	"	699,700	17.60	0.366
3C	"	40,400	1.01	.021
3D	"	32,600	----	----
3E	"	36,700	----	----
4A	205,200	2,112,200	10.29	.214
4B	"	2,637,800	12.85	.267
4C	"	121,700	----	----
4D	"	101,600	----	----
4E	"	106,900	----	----
5A	32,300	1,251,500	38.74	0.807
5B	"	816,000	25.26	0.526
5C	"	21,100	----	----
5D	"	17,000	----	----
5E	"	12,000	----	----
6A	38,200	607,900	15.91	.331
6B	"	159,000	4.16	.086
6C	"	66,400	1.73	.036
6D	"	38,100	----	----
6E	"	38,700	1.01	.021

TABLE 8

Chlorophyl a

Experiment J

Strain	Day	Chlor. a mg/l.	Strain	Day	Chlor. a mg/l.	Strain	Day	Chlor. a mg/l.
1A	0	40.08	2C	0	19.2	4A	0	---
	5	130.96		5	24.8		5	61.6
	15	65.28		15	70.2		15	221.0
	30	719.60		30	222.4		30	684.0
	45	707.36		45	568.8		45	863.8
	60	737.92		60	880.2		60	1291.4
1B	0	50.08	2E	0	16.8	4C	0	37.0
	5	62.16		5	39.0		5	39.4
	15	274.00		15	68.4		15	568.6
	30	726.16		30	203.4		30	1210.2
	45	695.12		45	437.8		45	498.2
	60	673.52		60	563.0		60	552.8
1C	0	62.48	3A	0	16.2	4E	0	44.8
	5	45.12		5	27.8		5	47.8
	15	298.00		15	38.8		15	133.6
	30	635.12		30	35.6		30	641.8
	45	908.56		45	58.6		45	1404.2
	60	351.60		60	47.4		60	1002.4
1D	0	39.60	3C	0	17.2	5A	0	---
	5	88.80		5	38.2		5	153.2
	15	289.68		15	41.6		15	102.2
	30	1105.52		30	50.4		30	338.4
	45	487.76		45	44.8		45	241.0
	60	551.12		60	22.0		60	568.8
1E	0	58.40	3E	0	4.6	5C	0	53.2
	5	107.36		5	16.8		5	59.2
	15	237.68		15	13.2		15	65.8
	30	789.36		30	17.2		30	119.0
	45	680.96		45	8.8		45	62.2
	60	521.12		60	21.4		60	91.0
2A	0	19.2				5E	0	53.2
	5	27.4					5	83.0
	15	74.2					15	85.0
	36	228.8					30	102.4
	45	693.2					45	77.8
	60	799.6					60	177.8

TABLE 9

Population Volume

Experiment J

Strain	Day	Pop Vol cc/l.	Strain	Day	Pop Vol cc/l.	Strain	Day	Pop Vol cc/l.
1A	0	.33	2C	0	.33	4A	0	.33
	5	.67		5	.33		5	.33
	15	1.33		15	1.33		15	1.33
	30	3.67		30	1.67		30	1.67
	45	4.67		45	2.67		45	3.33
	60	4.67		60	4.33		60	3.33
1B	0	.33	2E	0	.33	4C	0	.33
	5	.67		5	.33		5	.67
	15	1.33		15	1.00		15	3.67
	30	3.33		30	1.33		30	3.00
	45	5.33		45	2.67		45	4.67
	60	4.33		60	3.33		60	4.33
1C	0	.33	3A	0	.33	4E	0	.33
	5	.67		5	.33		5	.67
	15	2.00		15	1.00		15	1.33
	30	3.33		30	1.00		30	4.00
	45	5.33		45	1.33		45	4.00
	60	5.33		60	1.33		60	5.33
1D	0	.33	3C	0	.33	5A	0	.67
	5	.67		5	.67		5	1.00
	15	2.33		15	1.33		15	.67
	30	3.33		30	1.33		30	3.00
	45	3.33		45	1.23		45	3.33
	60	3.33		60	1.33		60	4.33
1E	0	.33	3E	0	.33	5C	0	.67
	5	.67		5	.67		5	1.00
	15	1.33		15	.67		15	4.00
	30	4.00		30	.67		30	5.00
	45	5.67		45	1.00		45	5.00
	60	6.67		60	1.00		60	5.33
2A	0	.33				5E	0	.67
	5	.33					5	.67
	15	1.00					15	.67
	36	1.23					30	2.67
	45	4.33					45	2.33
	60	4.33					60	4.00

TABLE 10 EXPERIMENT J

POPULATION DATA

(Phenol)

Strains & Pollutant conc.		Total cells (n/4)	2-cell coenobia (n/2)	4-cell coenobia (n/1)	8-cell coenobia (n x 2)
-1 $(Ac)_o$	units/ml	73,600	5,000	67,600	1,000
$(Ac)_o$	%	100	6.79	91.84	1.358
$(Ac)_f$	units/ml	2,241,000	413,000	1,684,000	144,000
$(Ac)_f$	%	100	18.42	75.14	6.42
B	units/ml	2,691,000	529,000	1,948,000	214,000
B	%	100	19.65	72.38	7.95
C	units/ml	2,728,500	504,500	2,076,000	148,000
C	%	100	18.49	76.08	5.42
D	units/ml	2,389,000	155,000	2,234,000	0
D	%	100	6.49	93.51	0
E	units/ml	2,477,500	385,500	1,928,000	164,000
E	%	100	15.56	77.82	6.61
-2 $(Ac)_o$	units/ml	42,000	34,000	8,000	0
$(Ac)_o$	%	100	80.95	19.04	0
$(Ac)_f$	units/ml	1,933,500	467,500	1,430,000	36,000
$(Ac)_f$	%	100	24.17	73.95	1.86
C	units/ml	1,378,500	322,500	1,052,000	4,000
C	%	100	23.39	76.31	0.29
E	units/ml	1,060,000	241,000	317,000	2,000
E	%	100	22.73	77.07	0.18
-3 $(Ac)_o$	units/ml	38,000	36,000	2,000	0
$(Ac)_o$	%	100	94.73	5.26	0
$(Ac)_f$	units/ml	158,500	141,500	17,000	0
$(Ac)_f$	%	100	89.27	10.72	0
C	units/ml	152,500	134,500	18,000	0
C	%	100	88.19	11.80	0
E	units/ml	386,000	302,000	84,000	0
E	%	100	78.23	21.76	0
-4 $(Ac)_o$	units/ml	86,100	2,100	82,000	2,000
$(Ac)_o$	%	100	2.43	95.23	2.32
$(Ac)_f$	units/ml	1,841,000	257,000	1,530,000	54,000
$(Ac)_f$	%	100	13.95	83.10	2.93
C	units/ml	1,711,000	30,000	1,157,000	524,000
C	%	100	1.75	67.62	30.62
E	units/ml	1,546,000	114,000	1,314,000	118,000
E	%	100	7.37	84.99	7.63
-5 $(Ac)_o$	units/ml	82,600	2,600	76,000	4,000
$(Ac)_o$	%	100	3.14	92.00	4.84
$(Ac)_f$	units/ml	966,500	138,500	798,000	30,000
$(Ac)_f$	%	100	14.33	82.56	3.10
C	units/ml	738,000	58,000	664,000	16,000
C	%	100	7.85	89.97	2.16
E	units/ml	549,000	78,000	453,000	18,000
E	%	100	14.20	82.51	3.27

TABLE 11 EXPERIMENT K

POPULATION DATA

($CuSO_4$)

Strains & Pollutant conc.		Total cells (n/4)	2-cell coenobia (n/2)	4-cell coenobia (n/1)	8-cell coenobia (n x 2)
-1 $(Ac)_o$	units/ml	97,500	41,500	44,000	12,000
$(Ac)^o$	%	100	42.56	45.12	12.30
$(Ac)^o_f$	units/ml	1,092,000	259,000	537,000	296,000
$(Ac)^f_f$	%	100	23.71	49.17	27.10
B	units/ml	725,500	105,500	460,000	160,000
B	%	100	14.54	63.40	22.05
C	units/ml	940,500	154,500	540,000	246,000
C	%	100	16.42	57.41	26.15
D	units/ml	1,027,000	118,000	627,000	282,000
D	%	100	11.48	61.05	27.45
E	units/ml	*	*	*	*
E	%	*	*	*	*
-2 $(Ac)_o$	units/ml	59,500	37,500	22,000	0
$(Ac)^o$	%	100	63.02	36.97	0
$(Ac)^o_f$	units/ml	159,500	123,500	36,000	0
$(Ac)^f_f$	%	100	77.42	22.57	0
B	units/ml	160,000	133,000	27,000	0
B	%	100	83.12	16.87	0
C	units/ml	271,500	218,500	53,000	0
C	%	100	80.47	19.52	0
D	units/ml	350,500	254,500	96,000	0
D	%	100	72.61	27.38	0
E	units/ml	*	*	*	*
E	%	*	*	*	*
-4 $(Ac)_o$	units/ml	72,000	44,000	28,000	0
$(Ac)^o$	%	100	61.11	38.88	0
$(Ac)^o_f$	units/ml	887,500	89,500	732,000	66,000
$(Ac)^f_f$	%	100	10.08	82.47	7.43
B	units/ml	1,564,000	408,000	884,000	272,000
B	%	100	26.08	56.52	17.17
C	units/ml	1,263,000	252,000	793,000	218,000
C	%	100	19.95	62.78	17.26
D	units/ml	1,001,500	171,500	624,000	206,000
D	%	100	17.12	62.30	20.56
E	units/ml	*	*	*	*
E	%	*	*	*	*

*Population minimal: haemacytometer counts not made.

TABLE 12 EXPERIMENT L

POPULATION DATA
(Biodegradable Detergent)

Strains & Pollutant conc.		Total cells (n/4)	2-cell coenobia (n/2)	4-cell coenobia (n/1)	8-cell coenobia (n x 2)
-1 $(Ac)_o$	units/ml	48,000	21,000	27,000	0
$(Ac)_o$	%	100	43.75	56.25	0
$(Ac)_f$	units/ml	193,000	9,000	182,000	2,000
$(Ac)_f$	%	100	4.66	94.30	1.03
B	units/ml	274,000	69,000	193,000	12,000
B	%	100	25.18	70.43	4.37
C	units/ml	180,000	7,000	171,000	2,000
C	%	100	3.88	95.00	1.11
D	units/ml	169,000	96,000	59,000	14,000
D	%	100	56.80	34.91	8.25
E	units/ml	*	*	*	*
E	%	*	*	*	*
-2 $(Ac)_o$	units/ml	65,000	34,000	29,000	2,000
$(Ac)_o$	%	100	52.30	44.61	3.07
$(Ac)_f$	units/ml	489,000	134,000	355,000	0
$(Ac)_f$	%	100	27.40	72.59	0
B	units/ml	1,509,000	301,000	1,201,000	7,000
B	%	100	19.94	79.58	0.46
C	units/ml	682,000	190,000	480,000	12,000
C	%	100	27.85	70.38	1.75
D	units/ml	1,181,000	439,000	721,000	21,000
D	%	100	37.17	61.04	1.77
E	units/ml	*	*	*	*
E	%	*	*	*	*

* Population minimal: hemacytometer counts not made

TABLE 12 EXPERIMENT L

POPULATION DATA
(Biodegradable Detergent)

Strains & Pollutant conc.		Total cells (n/4)	2-cell coenobia (n/2)	4-cell coenobia (n/1)	8-cell coenobia (n x 2)
-4 $(Ac)_o$	units/ml	72,000	38,000	34,000	0
$(Ac)_o$	%	100	52.77	47.22	0
$(Ac)_f$	units/ml	504,000	37,000	436,000	31,000
$(Ac)_f$	%	100	7.34	86.50	6.15
B	units/ml	259,000	26,000	216,000	17,000
B	%	100	10.03	83.39	6.56
C	units/ml	339,000	139,000	164,000	36,000
C	%	100	41.00	48.37	10.61
D	units/ml	277,000	154,000	98,000	25,000
D	%	100	55.59	35.37	9.02
E	units/ml	*	*	*	*
E	%	*	*	*	*

* Population minimal: hemacytometer counts not made

TABLE 13 EXPERIMENT M

POPULATION DATA

(Phenol)

Strains & Pollutant conc.			Total cells (n/4)	2-cell coenobia (n/2)	4-cell coenobia (n/1)	8-cell coenobia (n x 2)
-1	$(Ac)_o$	units/ml	217,300	1,400	186,600	29,300
	$(Ac)_o$	%	100	0.64	85.87	13.48
	$(Ac)_f$	units/ml	1,834,400	37,200	1,727,500	69,700
	$(Ac)_f$	%	100	2.02	94.17	3.69
	B	units/ml	1,857,900	132,600	1,602,700	122,600
	B	%	100	7.13	86.26	6.59
	C	units/ml	846,900	8,900	678,000	160,000
	C	%	100	1.05	80.05	18.89
	D	units/ml	95,500	35,900	59,600	0
	D	%	100	37.59	62.40	0
-2	$(Ac)_o$	units/ml	320,400	13,400	306,400	600
	$(Ac)_o$	%	100	4.18	95.63	0.18
	$(Ac)_f$	units/ml	1,613,300	149,300	1,434,700	29,300
	$(Ac)_f$	%	100	9.25	88.92	1.81
	B	units/ml	2,021,900	234,800	1,722,200	64,900
	B	%	100	11.61	85.17	3.20
	C	units/ml	634,100	36,100	560,700	37,300
	C	%	100	5.69	88.42	5.88
	D	units/ml	22,800	4,400	18,400	0
	D	%	100	19.29	80.70	0
-4	$(Ac)_o$	units/ml	273,300	4,300	246,200	22,800
	$(Ac)_o$	%	100	1.57	90.08	8.34
	$(Ac)_f$	units	884,900	96,900	528,000	260,000
	$(Ac)_f$	%	100	10.95	59.66	29.38
	B	units/ml	2,027,000	57,200	1,656,000	313,800
	B	%	100	2.82	81.69	15.48
	C	units/ml	570,300	24,900	434,700	110,700
	C	%	100	4.36	76.22	19.41
	D	units/ml	31,000	200	29,300	1,500
	D	%	100	0.64	94.51	4.83
-5	$(Ac)_o$	units/ml	24,200	0	8,200	16,000
	$(Ac)_o$	%	100	0	33.88	66.11
	$(Ac)_f$	units/ml	333,300	33,700	235,600	64,000
	$(Ac)_f$	%	100	10.11	70.68	19.20
	B	units/ml	449,000	52,300	346,900	49,800
	B	%	100	11.64	77.26	11.09
	C	units/ml	211,300	7,200	142,700	61,400
	C	%	100	3.40	67.53	29.05
	D	units/ml	9,300	100	6,800	2,400
	D	%	100	1.07	73.11	25.80

TABLE 14 EXPERIMENT N

POPULATION DATA

($CuSO_4$) after 46 days

Strains & Pollutant conc.		Total cells (n/4)	2-cell coenobia (n/2)	4-cell coenobia (n/1)	8-cell coenobia (n x 2)
-1 $(Ac)_o$	units/ml	217,300	1,400	186,600	29,300
$(Ac)_o$	%	100	.64	85.87	13.48
$(Ac)_f$	units/ml	1,922,700	70,700	1,764,000	88,000
$(Ac)_f$	%	100	3.67	91.74	4.57
B	units/ml	1,655,300	87,300	1,554,700	13,300
B	%	100	5.27	93.92	0.80
C	units/ml	1,501,200	25,300	1,449,300	26,600
C	%	100	1.68	96.54	1.77
D	units/ml	832,350	18,700	762,670	51,000
D	%	100	2.24	91.62	6.12
E	units/ml	85,770	17,100	68,670	0
E	%	100	19.93	80.06	0
-2 $(Ac)_o$	units/ml	320,400	13,400	306,400	600
$(Ac)_o$	%	100	4.18	95.63	0.18
$(Ac)_f$	units/ml	785,900	59,300	673,300	53,280
$(Ac)_f$	%	100	7.54	85.67	6.77
B	units/ml	935,200	51,300	854,600	29,300
B	%	100	5.48	91.38	3.13
C	units/ml	429,400	34,700	392,000	2,700
C	%	100	8.08	91.29	0.62
D	units/ml	100,000			
D	%	100			
E	units/ml	50,000			
E	%	100			
-3 $(Ac)_o$	units/ml	148,200	12,700	135,200	300
$(Ac)_o$	%	100	8.56	91.22	00.20
$(Ac)_f$	units/ml	1,554,000	628,700	925,300	0
$(Ac)_f$	%	100	40.45	59.54	0
B	units/ml	1,514,700	1,022,700	492,000	0
B	%	100	67.51	32.48	0
C	units/ml	982,700	305,300	677,300	0
C	%	100	31.06	68.92	0
D	units/ml	382,700	228,000	154,700	0
D	%	100	59.57	40.42	0
E	units/ml	22,000	6,200	15,800	0
E	%	100	28.18	71.81	0

TABLE 14 EXPERIMENT N
(continued)

Strains & Pollutants		Total cells (n/4)	2-cell coenobia (n/2)	4-cell coenobia (n/1)	8-cell coenobia (n x 2)
-5 $(Ac)_o$	units/ml	24,200	0	8,200	16,000
$(Ac)_o$	%	100	0	33.88	66.11
$(Ac)_f$	units/ml	370,700	30,000	271,800	68,900
$(Ac)_f$	%	100	8.09	73.32	18.58
B	units/ml	476,600	60,000	350,900	65,700
B	%	100	12.58	73.62	13.78
C	units/ml	253,500	30,400	161,100	62,000
C	%	100	11.99	63.55	24.45
D	units/ml	129,500	2,200	110,900	16,400
D	%	100	1.69	85.63	12.66
E	units/ml	10,600	100	8,300	2,200
E	%	100	0.94	78.30	20.75

TABLE 15 EXPERIMENT O

POPULATION DATA
(Phenol)

Strains & Pollutant conc.		Total cells (n/4)	2-cell coenobia (n/2)	4-cell coenobia (n/1)	8-cell coenobia (n x 2)
-1 $(Ac)_o$	units/ml	93,000	1,400	87,900	3,700
$(Ac)_o$	%	100	1.51	94.52	3.97
$(Ac)_f$	units/ml	3,123,100	150,700	2,922,700	49,700
$(Ac)_f$	%	100	4.82	93.59	1.59
B	units/ml	3,229,400	18,300	3,168,900	42,200
B	%	100	0.56	98.13	1.31
C	units/ml	2,196,000	19,400	2,134,400	42,200
C	%	100	0.88	97.19	1.93
D	units/ml	107,200	2,100	103,100	2,000
D	%	100	1.95	96.18	1.87
E	units/ml	104,500	1,700	99,700	3,100
E	%	100	1.63	95.41	2.96
-2 $(Ac)_o$	units/ml	119,500	12,100	107,400	0
$(Ac)_o$	%	100	10.12	89.88	0
$(Ac)_f$	units/ml	3,353,700	301,900	3,011,800	40,000
$(Ac)_f$	%	100	9.00	89.80	1.20
B	units/ml	2,134,400	124,400	1,925,600	84,400
B	%	100	5.82	90.22	3.96
C	units/ml	1,934,700	123,800	1,777,700	33,200
C	%	100	6.39	91.89	1.72
D	units/ml	99,600	10,000	89,600	0
D	%	100	10.04	89.96	0
E	units/ml	110,400	9,300	101,100	0
E	%	100	8.43	91.57	0

TABLE 15 EXPERIMENT O

POPULATION DATA

Blue Series I = Phenol

Strains & Pollutant conc.		Total cells (n/4)	2-cell coenobia (n/2)	4-cell coenobia (n/1)	8-cell coenobia (n x 2)
-3 $(Ac)_o$	units/ml	39,700	28,600	11,100	0
$(Ac)_o$	%	100	72.05	27.95	0
$(Ac)_f$	units/ml	1,008,300	358,600	649,700	0
$(Ac)_f$	%	100	35.56	64.44	0
B	units/ml	955,500	371,700	582,200	1,600
B	%	100	38.90	60.94	0.16
C	units/ml	64,100	36,000	28,000	100
C	%	100	56.16	43.68	0.16
D	units/ml	42,600	31,500	11,100	0
D	%	100	73.95	26.05	0
E	units/ml	37,100	27,100	10,000	0
E	%	100	73.05	26.95	0
-4 $(Ac)_o$	units/ml	176,300	118,500	57,800	0
$(Ac)_o$	%	100	67.22	32.78	0
$(Ac)_f$	units/ml	1,609,700	527,500	927,800	154,400
$(Ac)_f$	%	100	32.77	57.64	9.59
B	units/ml	2,104,200	713,300	1,155,500	235,400
B	%	100	33.89	54.92	11.19
C	units/ml	2,173,400	906,700	1,080,000	186,700
C	%	100	41.72	49.69	8.59
D	units/ml	226,600	104,400	122,200	0
D	%	100	46.07	53.93	0
E	units/ml	174,100	87,900	86,200	0
E	%	100	50.48	49.52	0

TABLE 15 EXPERIMENT O
(Phenol)

Strains & Pollutant conc.		Total cells (n/4)	2-cell coenobia (n/2)	4-cell coenobia (n/1)	8-cell coenobia (n x 2)
-5 $(Ac)_o$	units/ml	32,300	4,200	27,900	200
$(Ac)_o$	%	100	13.00	86.37	0.63
$(Ac)_f$	units/ml	1,251,600	88,000	1,096,500	67,100
$(Ac)_f$	%	100	7.03	87.61	5.36
B	units/ml	888,500	32,500	842,700	13,300
B	%	100	3.66	94.85	1.49
C	units/ml	451,100	10,000	420,000	21,100
C	%	100	2.21	93.11	4.68
D	units/ml	27,500	3,200	24,100	200
D	%	100	11.63	87.64	0.73
E	units/ml	29,100	3,100	25,800	200
E	%	100	10.65	88.66	0.69
-6 $(Ac)_o$	units/ml	30,200	10,600	19,600	0
$(Ac)_o$	%	100	35.09	64.90	0
$(Ac)_f$	units/ml	616,400	37,100	570,400	8,900
$(Ac)_f$	%	100	6.01	92.53	1.44
B	units/ml	584,900	35,500	548,300	1,100
B	%	100	6.06	93.74	0.18
C	units/ml	93,700	26,600	66,200	900
C	%	100	28.38	70.65	0.96
D	units/ml	29,100	11,800	17,300	0
D	%	100	40.54	59.45	0
E	units/ml	28,700	13,300	15,400	0
E	%	100	46.34	53.65	0

TABLE 16 EXPERIMENT P

POPULATION DATA
($CuSO_4$)

Strain & Pollutant conc.		Total cells (n/4)	2-cell coenobia (n/2)	4-cell coenobia (n/1)	8-cell coenobia (n x 2)
-1 $(Ac)_o$	units/ml	93,000	1,400	87,900	3,700
$(Ac)_o$	%	100	1.51	94.52	3.97
$(Ac)_f$	units/ml	3,123,200	150,700	2,922,700	49,800
$(Ac)_f$	%	100	4.82	93.59	1.59
B	units/ml	1,448,900	43,900	1,401,700	3,300
B	%	100	3.02	96.75	0.23
C	units/ml	95,700	500	91,200	4,000
C	%	100	0.53	95.30	4.17
D	units/ml	104,100	2,000	97,400	4,700
D	%	100	1.93	93.58	4.49
E	units/ml	91,800	1,900	85,900	4,000
E	%	100	2.06	93.58	4.36
-2 $(Ac)_o$	units/ml	119,500	12,100	107,400	0
$(Ac)_o$	%	100	10.12	89.87	0
$(Ac)_f$	units/ml	3,353,800	301,900	3,011,800	40,100
$(Ac)_f$	%	100	9.00	89.81	1.19
B	units/ml	1,495,800	253,300	1,217,300	25,200
B	%	100	16.94	81.38	1.68
C	units/ml	1,016,500	76,300	938,000	2,200
C	%	100	7.51	92.27	0.22
D	units/ml	443,300	172,000	270,400	900
D	%	100	38.80	60.99	0.21
E	units/ml	305,200	104,900	200,300	0
E	%	100	34.38	65.62	0

TABLE 16 EXPERIMENT P
($CuSO_4$)

Strain & Pollutant conc.		Total cells (n/4)	2-cell coenobia (n/2)	4-cell coenobia (n/1)	8-cell coenobia (n x 2)
-3 $(Ac)_o$	units/ml	39,700	28,600	11,100	0
$(Ac)_o$	%	100	72.04	27.96	0
$(Ac)_f$	units/ml	1,008,300	358,600	649,700	0
$(Ac)_f$	%	100	35.56	64.44	0
B	units/ml	699,700	335,000	364,700	0
B	%	100	47.87	52.13	0
C	units/ml	40,400	29,600	10,800	0
C	%	100	73.27	26.73	0
D	units/ml	32,600	24,400	8,200	0
D	%	100	74.84	25.16	0
E	units/ml	36,700	29,100	7,600	0
E	%	100	79.29	20.71	0
-4 $(Ac)_o$	units/ml	205,200	147,400	57,800	0
$(Ac)_o$	%	100	71.83	28.17	0
$(Ac)_f$	units/ml	2,112,200	1,030,000	927,800	154,400
$(Ac)_f$	%	100	48.76	43.93	7.31
B	units/ml	2,637,800	985,900	1,543,700	108,200
B	%	100	37.37	58.52	4.11
C	units/ml	121,700	80,400	41,100	200
C	%	100	66.06	33.78	0.16
D	units/ml	101,600	74,400	27,200	0
D	%	100	73.22	26.78	0
E	units/ml	106,900	74,800	32,100	0
E	%	100	69.98	30.02	0

TABLE 16 EXPERIMENT P
($CuSO_4$)

Strains & Pollutant conc.		Total cells (n/4)	2-cell coenobia (n/2)	4-cell coenobia (n/1)	8-cell coenobia (n x 2)
-5 $(Ac)_o$	units/ml	32,300	4,200	27,900	200
$(Ac)_o$	%	100	13.00	86.37	0.61
$(Ac)_f$	units/ml	1,251,500	88,000	1,096,400	67,100
$(Ac)_f$	%	100	7.03	87.60	5.36
B	units/ml	816,000	204,000	600,000	12,000
B	%	100	25.00	73.52	1.47
C	units/ml	21,100	2,100	18,800	200
C	%	100	9.95	89.09	0.94
D	units/ml	17,000	1,400	15,200	400
D	%	100	8.23	89.41	2.35
E	units/ml	12,000	1,300	10,500	200
E	%	100	10.83	87.50	1.66
-6 $(Ac)_o$	units/ml	38,200	10,600	19,600	8,000
$(Ac)_o$	%	100	27.74	51.30	20.94
$(Ac)_f$	units/ml	607,900	37,100	570,400	400
$(Ac)_f$	%	100	6.10	93.83	0.06
B	units/ml	159,000	37,800	121,200	0
B	%	100	23.77	76.22	0
C	units/ml	66,400	29,500	36,900	0
C	%	100	44.42	55.57	0
D	units/ml	38,100	15,900	22,200	0
D	%	100	41.73	58.26	0
E	units/ml	38,700	12,600	26,100	0
E	%	100	32.54	67.42	0

TABLE 17

PHENOL SUMMARY SHEET
Diel Increase Factors

	Experimental Code					Experimental Code			
Strain 1	J	M	O	Mean	Strain 2	J	M	O	Mean
ppm									
0.0	.51	.18	.70	.46		.77	.11	.58	.49
0.1	.61								
1.0	.60					.55			.55
10.0	.54	.18		.36			.14		.14
100.0	.56	.08	.74	.46		.42	.04	.37	.27
250.0			.47	.47				.33	.33
500.0			—	—				—	—
750.00			—	—				—	—
1000.0		—		—			—		—

Strain 3	J	M	O	Mean	Strain 4	J	M	O	Mean
0.0	.07		.52	.29		.36	.07	.09	.17
0.1									
1.0	.07			.07		.34			.34
10.0							.16		.16
100.0	.17		.50	.33		.30	.04	.12	.15
250.0			.03	.03				.16	.16
500.0			.02	.02				—	—
750.0			—					—	—
1000.0							—		—

Strain 5	J	M	O		Strain 6	J	M	O	Mean
0.0	.19	.30	.81	.43				.42	.42
0.1									
1.0	.15			.15					
10.0		.40		.40					
100.0	.11	.19	.57	.29				.40	.40
250.0			.29	.29				.06	.06
500.0			—	—				—	—
750.0			—	—				—	—
1000.0		—		—					

TABLE 18

$CuSO_4$ SUMMARY SHEET

Diel Increase Factors

Strain 1	Experimental Code				Strain 2	Experimental Code			
ppm	K	N	P	Mean		K	N	P	Mean
0.0	0.40	.192	.699	.430		0.09	.053	.584	.242
0.1	0.23	.165		.197		0.06	.063		.061
1.0	0.32	.150		.235		0.10	.029		.064
10.0	0.37	.083	.324	.288		0.13	.006	.260	.132
25.0			.021	.021				.177	.177
50.0			.026	.026				.077	.077
75.0			—	—				.053	.053
100.00	—	—		—		—	—		

Strain 3				Strain 4			
0.0	.227	.528	.377	0.45	.273	.214	.312
0.1	.222		.222	0.55	.260		.405
1.0	.144		.144	0.34	.217		.278
10.0	.056	.366	.211	0.26	.139	.267	.222
25.0		.021	.021			—	—
50.0		—	—			—	—
75.0		—	—			—	—
100.0	—		—	—	—		

Strain 5				Strain 6		
0.0	.332	.807	.569		.331	.331
0.1	.428		.428			
1.0	.227		.227			
10.0	.116	.526	.321		.086	.086
25.0		—	—		.036	.036
50.0		—	—		—	—
75.0		—	—		.021	.021
100.0	—		—			

TABLE 19 --CELLS PER COENOBIUM*

AS % OF POPULATION

Strain	Experiment Code	2-celled % range	4-celled % range	8-celled % range
1	J	6.4-19.6	72.3-93.5	0- 7.9
	K	11.4-42.5	45.1-63.4	0-27.4
	L	3.8-56.8	34.9-95.0	0- 8.2
	M	0.6-37.5	62.4-94.1	0-13.4
	N	0.6-19.9	80.0-96.5	0-13.4
	O	0.5- 4.8	93.5-98.1	1.3- 3.9
	P	0.5- 4.8	93.5-96.7	0.2- 4.4
2	J	22.7-80.9	19.0-77.0	0- 1.8
	K	63.0-83.1	16.8-36.9	0
	L	19.9-52.3	44.6-79.5	0- 3.0
	M	4.1-19.2	80.7-95.6	0- 5.8
	N	4.1- 8.0	85.6-95.6	0.1- 6.7
	O	5.8-10.1	89.8-91.8	0- 3.9
	P	7.5-38.8	60.9-92.2	0- 1.6
3	J	78.2-94.7	5.2-21.7	0
	N	8.5-67.5	32.4-91.2	0- 0.2
	O	35.5-73.9	26.0-64.4	0- 0.1
	P	35.5-79.2	20.7-64.4	0
4	J	1.7-13.9	67.6-95.2	2.3-30.6
	K	10.0-61.1	38.8-82.4	0-20.5
	L	7.3-55.5	35.3-86.5	0-10.6
	M	0.6-10.9	59.6-94.5	4.8-29.3
	O	33.8-67.2	32.7-57.6	0-11.1
	P	37.3-73.2	26.7-58.5	0- 7.3
5	J	3.1-14.3	82.5-92.0	2.1- 4.8
	M	0.0-11.6	33.8-77.2	11.0-66.1
	N	0.0-12.5	33.8-85.6	12.6-66.1
	O	2.2-13.0	86.3-94.8	0.6- 5.3
	P	7.0-25.0	73.5-89.4	0.6- 5.3
6	O	6.0-46.3	53.6-93.7	0- 1.4
	P	6.1-44.4	51.3-93.8	0-20.9

*i.e. percent of total cell no. contained resp. in 2-celled, 4-celled and 8-celled coenobia.